Alan Lichtin
John Bartholomew
Editors

The Coagulation Consult

A Case-Based Guide

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For J. Leon and Beverly Lichtin

—Alan Lichtin

For Kathleen Bartholomew

—John Bartholomew

Preface

The reader might ask, "Why does the world need another coagulation textbook?" In this time of instant access to medical information on the Internet, indeed, one might ask what is the worth of any textbook, with its inherent publication delay.

Many texts in the field of coagulation lean toward an emphasis on basic science. This text does not do that. The goal of this book is to describe clinical scenarios for which the practicing hematologist or vascular medicine expert (either vascular medicine doctor or vascular surgeon) is consulted for bleeding or clotting issue.

Many of us are very comfortable dealing with the spectrum of bleeding and clotting disorders, and yet, these days, many of us feel more comfortable dealing with one or the other. In fact, at many institutions, there are separate departments of hematology (often overly weighted to the malignant hematology side) and vascular medicine/vascular surgery. The bleeding patients tend to be seen by the hematologists, and the thrombotic patients are more frequently evaluated and treated by the vascular medicine doctors.

There are several disorders that present challenges such that both teams are called to the bedside, and cooperation between these two services leads to the best results. This is especially true for the heparin-associated thrombocytopenia (HIT) patients, who do not recover their platelet counts as one might expect. They may remain on a direct thrombin inhibitor, and day after day, the platelets remain frustratingly low. The vascular medicine doctors will call the hematologists to make sure that there is not some other reason for the thrombocytopenia. Likewise, the severely affected antiphospholipid patient may present with thrombocytopenia and be seen by the hematologists first, and the thrombotic aspect of the disorder will be of more paramount importance, and the hematologist may call the vascular medicine colleague to help. Another common scenario where one service calls the other is when there is a patient with a thrombosis in an unusual location and is first seen by the vascular medicine doctor and work-up suggests a primary hematologic reason for the thrombosis, such as a myeloproliferative disorder or paroxysmal nocturnal hemoglobinuria. That is when the hematologist might be called.

This book is divided into chapters whose titles are the typical reasons we are consulted to see patients. Our non-hematologic colleagues will call us for a patient with a prolonged PT, a prolonged PTT, bleeding with surgery, easy bruising, etc. The reader should look over the chapter headings and realize

that many of the reasons we are consulted are listed there. Also, chapters are devoted to special categories of patients, such as the patient with postoperative bleeding, the patient with thrombosis around catheters, the individual with heparin-induced thrombocytopenia, and the pregnant woman.

We wish to acknowledge many individuals who have made this text possible. The team of editors at Springer, especially Michael Wilt, have been most helpful. The photography in the chapter on Easy Bruising was made possible by Janine Sot. This book obviously could not have been written without the help of our authors, and we appreciate their efforts. Also, we have been blessed to have an exceptional secretary, Marge Dvorsack, to prepare the manuscripts for the publisher. She has done a phenomenal job.

Cleveland, OH

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Contents

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Laboratory Analysis of Coagulation

1

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List of Abbreviations

AA	Arachidonic acid
ACA	Anticardiolipin antibody
ADP	Adenosine diphosphate
APA	Antiphospholipid antibody
APC	Activated protein C
APC-R	APC resistance
APS	Antiphospholipid syndrome
aPTT	Activated partial thromboplastin time
AR	Autosomal recessive
AS	Allele-specific
ASA	Aspirin (acetyl salicylic acid)
AT	Antithrombin
ATP	Adenosine triphosphate
B2GPI	Beta2 glycoprotein 1
BT	Bleeding time
BU	Bethesda unit
C4bBP	C4b-binding protein
CAP	College of American Pathologists
CLIA	Clinical Laboratory Improvement Amendments
COX1	Cyclooxygenase 1
CT	Closure time
DIC	Disseminated intravascular coagulation
DRVVT	Dilute Russell's viper venom test
DTI	Direct thrombin inhibitor
DVT	Deep vein thrombosis
ELISA	Enzyme-linked immunosorbent assay
ELT	Euglobulin lysis time
EM	Electron microscopy
ET	Essential thrombocythemia
FDP	Fibrin degradation product
FII	Prothrombin
FIIa	Thrombin
FVIIa	Activated factor VII
FVIII	Factor VIII
FVL	Factor V Leiden
FRET	Fluorescence resonance energy transfer
GP	Glycoprotein
HMWK	High-molecular-weight kininogen
INR	International normalized ratio
ISI	International sensitivity index
ISTH	International Society for Thrombosis and Haemostasis
LA	Lupus anticoagulant
LMW	Low molecular weight
MPN	Myeloproliferative neoplasms
MPV	Mean platelet volume

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MTHFR	Methylenetetrahydrofolate reductase
NSAIDs	Nonsteroidal anti-inflammatory drugs
PAI	Plasminogen activator inhibitor
PCR	Polymerase chain reaction
PDW	Platelet distribution width
PE	Pulmonary embolism
PFA	Platelet function analyzer
PK	Prekallikrein
PRP	Platelet rich plasma
PT	Prothrombin time
RFLP	Restriction fragment length polymorphism
RIPA	Ristocetin-induced platelet aggregation
RT	Reptilase time
SLE	Systemic lupus erythematosus
SNP	Single nucleotide polymorphism
TAFI	Thrombin-activatable fibrinolysis inhibitor
TAR	Thrombocytopenia with absent radii
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TM	Thrombomodulin
tPA	Tissue plasminogen activator
TT	Thrombin time
TxA_2	Thromboxane A_2
uPA	Urokinase plasminogen activators
VTE	Venous thromboembolism
VWD	von Willebrand disease
VWF	von Willebrand factor
XR	X-linked recessive

Introduction of Hemostasis and Thrombosis

The goal of physiologic hemostasis is to stop any bleeding that occurs and, ultimately, to return the vessel wall back to its original state. This is achieved through a dynamic interaction of pro- and anticoagulant elements. Early studies of hemostasis focused primarily on the process of clot formation. Originally described as a coagulation "cascade," the model for in vivo hemostasis subsequently evolved to incorporate the more complex contributions of elements beyond the traditional coagulation factors (Roberts et al. 1998; Hoffman and Monroe 2001; Schmaier and Miller 2011). Although it is now well established that the classic coagulation cascade does not accurately depict in vivo events, it remains particularly relevant with regard to understanding the in vitro process of hemostasis reflected by widely used coagulation screening tests such as the prothrombin time (PT) and activated partial thromboplastin time (aPTT).

Physiology of Hemostasis

Following an insult to the vascular wall, hemostasis is initiated by platelet adhesion at the site of injury. This is followed by platelet aggregation and degranulation, with release of multiple mediators and procoagulant factors by the activated platelets. At the same time, tissue factor expressed at the site of injury initiates serial activation of coagulation factors. These events culminate in the formation of a fibrin thrombus which incorporates the activated platelets into its structure. In order to prevent the clot from growing uncontrollably, antithrombotic mechanisms are activated to maintain the balance of pro- and anticoagulant processes. Clot remodeling by fibrinolysis occurs over time, while cellular elements move in to repair the underlying tissue damage. The remainder of the clot is eventually eliminated and vascular patency and integrity restored. Thrombin plays a key role in virtually every step of the hemostatic process. Derangements of one or more pro- or anticoagulant elements of hemostasis may result in an increased risk of bleeding, an increased risk of clotting, or, rarely, both.

Initiation of Hemostasis by Platelet Plug Formation

The role of platelets in hemostasis and laboratory evaluation of platelet function are discussed in section of this chapter.

Initiation and Propagation of Clotting Through Activation of Coagulation Factors

Clotting factors are proenzymes or inactive precursor proteins (zymogens), enzyme cofactors, and substrates that are sequentially activated to form a fibrin clot. All of these factors are made

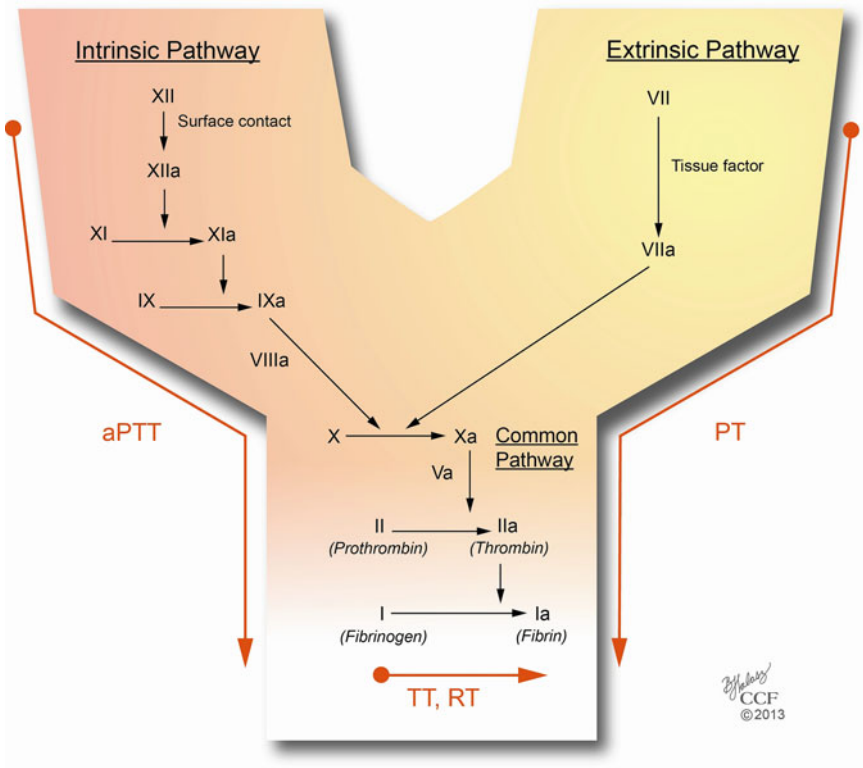

Fig. 1.1 Classic coagulation cascade. This model of coagulation depicts extrinsic, intrinsic, and common pathways of coagulation. Calcium ions and phospholipids, which are not depicted for simplicity, are necessary cofactors in several steps of this process (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2013. All Rights Reserved). *aPTT* activated partial thromboplastin time, *PT* prothrombin time, *TT* thrombin time, *RT* reptilase time

in the liver by hepatocytes, except for factor VIII (FVIII) which may be made by the reticuloendothelial system (Shovlin et al. 2010; Schmaier and Miller 2011). Some of the procoagulant factors (II, VII, IX, and X) undergo vitamin K-dependent gamma-carboxylation, which allows them to bind to the phospholipid surfaces where they are activated. Nutritional vitamin K deficiency and oral anticoagulation with warfarin, a vitamin K antagonist, anticoagulate by disrupting this process (Ageno et al. 2012; Schmaier and Miller 2011).

Classic Coagulation Cascade

The classic coagulation cascade (Fig. 1.1) illustrates intrinsic and extrinsic pathways of clotting which converge in a common pathway ending in clot formation. The extrinsic pathway, which is assessed by the PT, starts with activation of FVII by tissue factor (TF), followed by direct activation of the common pathway by activated factor VII (FVIIa). The intrinsic pathway, which is assessed by the aPTT, starts with activation of the contact factor XII, followed by a cascading activation of factors XI then IX. Activated factor VIII serves as a cofactor for activation of the common pathway by FIXa. Once the common pathway is initiated by activation of FX by either FVIIa or FIXa, activated factor V serves as a cofactor for FXa to activate prothrombin (FII) to thrombin (FIIa), which in turn cleaves fibrinogen (factor I) to fibrin. Calcium is a necessary cofactor for nearly all of the above steps, while phospholipid is required for activation events in the intrinsic pathway and for activation of FII (Mann 2003; Hoffman and Monroe 2007; Schmaier and Miller 2011; Leung 2013).

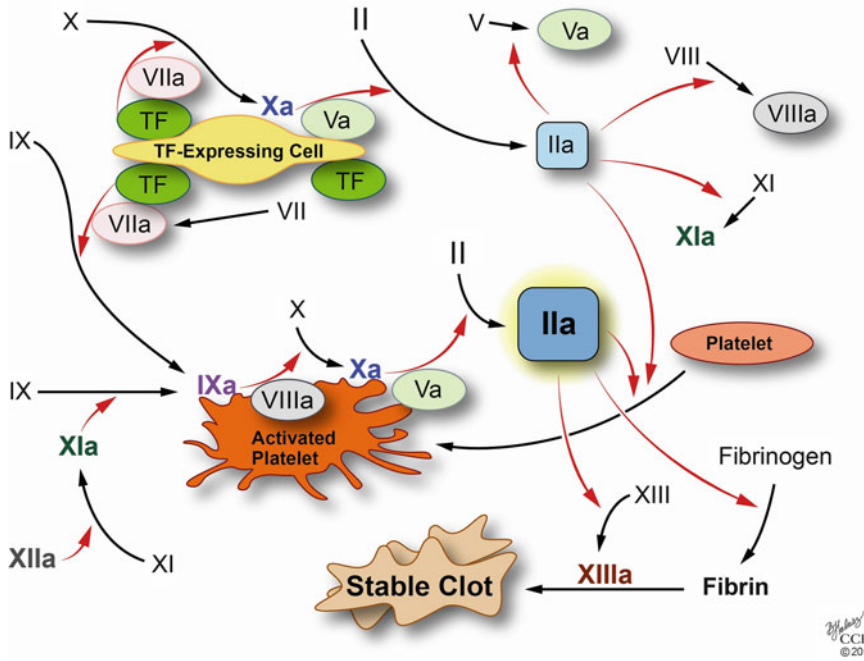

Fig. 1.2 Cell-based model of coagulation. This model of coagulation incorporates some of the cellular elements involved in coagulation and better reflects the complexity and interdependence of the elements of in vivo coagulation. Calcium ions, which are not depicted for simplicity, are necessary cofactors in several steps of this process. In the cell-based model of coagulation, FVII is activated to FVIIa by tissue factor (TF). The TF–FVIIa complex activates FX to FXa, which together with its cofactor FVa activates prothrombin (FII) to thrombin (FIIa). In addition to activating factors V, VIII, and XI, the FIIa generated by this mechanism also activates platelets. Factor IX is activated by both the TF–FVIIa complex and FXIa. Together with its cofactor FVIIIa, FIXa activates FX on the surface of activated platelets. FXa, together with its cofactor FVa, activates prothrombin (FII) to thrombin (FIIa). The thrombin (FIIa) generated at this point converts fibrinogen (FI) to fibrin (FIa) and factor XIII to the clot-stabilizing FXIIIa. The end result of this process is a stable, cross-linked polymerized fibrin clot. *Black arrows* indicate transition of inactivated factors to their activated forms. *Red arrows* indicate an activating effect, with cofactor contribution by the factors tangential to the *red arrows* (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2013. All Rights Reserved)

Cell-Based Model of Coagulation

Activation of circulating FVII to FVIIa by tissue factor is the primary initiator of the coagulation cascade in both the classic and cell-based models of coagulation. However, the cell-based model of coagulation better reflects the complexity and interdependence of the in vivo events resulting in clot formation (Fig. 1.2).

Tissue factor (TF) is a transmembrane glycoprotein expressed in a variety of cells which are not typically in direct contact with the blood flow, including vascular smooth muscle cells, adventitial fibroblasts, and pericytes. Endothelial cells do not normally express TF. In the event of an injury to a vascular wall resulting in endothelial damage or disruption, expression of TF is increased and TF-bearing cells are exposed to circulating blood. TF activates FVII to FVIIa (Rapaport and Rao 1995; Hoffman and Monroe 2007; Breitenstein et al. 2009; Leung 2013).

The TF–FVIIa complex activates FX to FXa. Activated factor V acts as a cofactor for FXa to activate prothrombin (FII) to thrombin (FIIa). The FVa needed for this process may be released directly from activated platelets or activated by FXa or non-coagulation proteases. The small amount of thrombin generated by TF–FVIIa activation stimulates upregulation of TF expression and activates platelets, resulting in exposure of the platelet phospholipid surfaces needed

for assembly of intrinsic factor activating complexes. Thrombin also directly activates factors V, VIII, and XI, which, together with activation of factor IX to FIXa by the TF–FVIIa complex, facilitates clotting through the intrinsic pathway. The hemostatic response is markedly amplified at this point given the ability of FIXa to diffuse to adjacent platelet surfaces, as opposed to the FXa generated by the TF–FVIIa complex which is localized to TF-expressing cell due to inhibition of FXa by antithrombin (AT) and tissue factor pathway inhibitor (TFPI) (Mann 2003; Hoffman and Monroe 2007; Schmaier and Miller 2011; Leung 2013).

Once activated, FVIIIa and FIXa quickly become localized to the surface of activated platelets and activate FX. The prothrombinase complex, consisting of FXa and its cofactor FVa, is a very potent thrombin generator in the common pathway. The enhanced thrombin generation by this mechanism results in conversion of fibrinogen to fibrin. The fibrin monomers undergo polymerization and the clot is then cross-linked and stabilized by FXIII, which is also activated by thrombin (Mann 2003; Hoffman and Monroe 2007; Schmaier and Miller 2011; Leung 2013).

FXII (Hageman factor) autoactivates in association with negatively charged surfaces, such as exposed collagen at the site of a vascular injury and polysomes released by activated platelets. FXII, prekallikrein (PK or Fletcher factor), and high-molecular-weight kininogen (HMWK, Fitzgerald, or Williams factor) comprise the contact system which can also activate FIX. The contact system factors are redundant in vivo; deficiencies in these factors are not associated with bleeding but may instead be associated with an increased risk of thrombosis (Gallimore et al. 2004; Girolami et al. 2011; Schmaier and Miller 2011).

Binding of thrombin to thrombomodulin (TM) induces a conformational change in thrombin whereby it ceases to activate platelets and cleave fibrinogen. The thrombin–TM complex activates protein C to decelerate the clotting process. In addition to its role in slowing down the clotting process, the thrombin–TM complex also activates thrombin-activatable fibrinolysis inhibitor (TAFI) to further stabilize the clot and protect it from rapid lysis by plasmin (Hoffman and Monroe 2007; Schmaier and Miller 2011; Leung 2013).

In summary, although the classic coagulation cascade might imply that the extrinsic and intrinsic pathways are redundant, the cell-based coagulation model makes it clear that they are not. Extrinsic pathway activities are limited to TF-expressing surfaces and result in initiation of clotting and activation of the platelets and coagulation factors needed for amplification of the hemostatic response. On the other hand, intrinsic pathway activities take place on the phospholipid surface of the activated platelets and generate the thrombin burst which facilitates formation and stabilization of the fibrin clot. Thus, the intrinsic and extrinsic coagulation pathways each play a unique and vital role in achieving hemostasis (Hoffman and Monroe 2007).

Termination of Clotting by Antithrombotic Mechanisms

The three main antithrombotic mechanisms involved in terminating clotting are tissue factor pathway inhibitor (TFPI), antithrombin (AT), and activated protein C (APC). Deficiencies of these natural anticoagulants, or their cofactors, can result in an increased risk of thrombosis. The function of natural anticoagulants is not captured by coagulation screening tests such as the PT and aPTT.

TFPI is the most potent inhibitor of TF–FVIIa complex and inhibits TF–FVIIa by forming a quaternary complex with FVIIa, TF, and FXa (Breitenstein et al. 2009; Leung 2013). Although AT inhibits most of the activated coagulation factors, including thrombin (FIIa) and factors IXa, Xa, XIa, and XIIa, it exerts its primary effect through inhibition of factors IIa and Xa. Endogenous and exogenous heparin and heparin-like substances can significantly potentiate the anticoagulant effect of AT, in some cases by 1,000-fold or greater (Schmaier and Miller 2011; Leung 2013). The structure of the different types of heparin plays a role in determining their effect through interaction with AT; for example, unfractionated heparin exerts its primary anticoagulant effect through AT-mediated inactivation of FIIa, whereas low molecular weight (LMW) heparins exert their primary anticoagulant effect through

AT-mediated inactivation of FXa (Garcia et al. 2012). The thrombin–TM complex activates protein C, which, in association with its cofactor protein S, inactivates factors Va and VIIIa. Activated protein C (APC) has also been found to play a role in other associated processes including inflammation and stimulating fibrinolysis (Schmaier and Miller 2011; Griffin et al. 2012; Leung 2013).

Clot Lysis

After hemostasis is achieved, it is important to remove the clot and restore the patency of the blood vessel as part of the wound healing process. Tissue plasminogen activator (tPA) converts fibrin-bound plasminogen to plasmin which cleaves the fibrin strands releasing fibrin degradation products (FDPs). D-dimer is a major FDP consisting of two D domains from adjacent fibrin monomers that had been cross-linked by FXIIIa. tPA is primarily responsible for initiating intravascular fibrinolysis, while urokinase plasminogen activators (uPA) perform this function in the extravascular compartment. Plasminogen activator inhibitors (PAI-1 and PAI-2) inhibit tPA and uPA, while alpha-2-antiplasmin inhibits plasmin (Hoffman and Monroe 2007; Schmaier and Miller 2011; Leung 2013). To further maintain the balance of pro- and antifibrinolytic processes, thrombin, plasmin, and the thrombin–TM complex may all activate thrombin-activatable fibrinolysis inhibitor (TAFI) to TAFIa, which inhibits fibrinolysis and protects the clot from premature degradation by plasmin (Hoffman and Monroe 2007; Schmaier and Miller 2011; Colucci and Semeraro 2012; Leung 2013).

The activity of pro- and antifibrinolytic factors is not captured by coagulation screening tests such as the PT and aPTT, or even by the thrombin time. The euglobulin lysis time (ELT) can be used as a screening test to assess global fibrinolytic function; assays for individual factors may also be performed. The ELT is expected to be shortened in hyperfibrinolysis and may be prolonged with hypofibrinolysis. However, the usefulness of ELT is limited by its relative insensitivity and a broad variation in results among normal individuals (Glassman et al. 1993).

Laboratory Assays for Evaluation of Coagulation Disorders

Commonly Used Laboratory Assays Related to Hemostasis

The most widely used screening tests of coagulation function are the prothrombin time (PT), the international normalized ratio (INR), and the activated partial thromboplastin time (aPTT). Unless otherwise specified, samples used for coagulation testing are collected in 3.2 % sodium citrate (light blue top) test tubes. The anticoagulant effect of citrate is exerted by chelating calcium, which is a required cofactor for most steps in the hemostatic process (Adcock et al. 1998).

Prothrombin Time (PT) and International Normalized Ratio (INR)

The PT assesses the extrinsic and common pathways (factors VII, X, V, II, and I) (Fig. 1.1). Patient plasma is incubated for a short time with thromboplastin, a source of phospholipid and tissue factor, and then recalcified. The PT is the time (in seconds) that it takes to form a fibrin clot after adding the calcium.

Variable prolongation of the PT was noted in patients on warfarin therapy depending on the thromboplastin reagent and test system used (Bailey et al. 1971). The INR was conceived to provide a standardized approach to therapeutic monitoring of warfarin, whereby an international sensitivity index (ISI) is determined by the manufacturer for each reagent/test system combination relative to the international standard for thromboplastin. The INR is then calculated as follows:

$$\text{INR} = (\text{Patient PT}/\text{Mean normal PT for the laboratory})^{\text{ISI}}$$

(Ageno et al. 2012). The INR has only been validated for patients on oral anticoagulant therapy with a vitamin K antagonist, in other words, those in whom only the vitamin K-dependent factors are expected to be decreased (Loeliger et al. 1985). The widespread adoption of the INR as a general indicator of coagulation function, and its incorporation into the model for end-stage liver disease (MELD) scoring system for prioritization

of patients for liver transplant, should be viewed with caution as the INR is not reliably reproducible across reagents, tests systems, and laboratories in settings other than vitamin K deficiency or antagonism. For example, patients with liver disease tested by different reagents or laboratories may have enough variation in their INR results to impact their MELD score without having had any significant change in their underlying condition (Sermon et al. 2010). In addition, utilizing the INR as a presumptive marker of coagulation hypofunction in patients with liver disease can be particularly misleading given the complexity of their underlying hemostatic derangements (Tripodi et al. 2011).

Also of note, as a PT-derived parameter, the INR may not be reliable for monitoring warfarin therapy in patients with lupus anticoagulants that are associated with a baseline prolongation of the PT. Although PT reagents are typically selected to be insensitive to the effect of lupus anticoagulants, some lupus anticoagulants and antiphospholipid antibodies with antiprothrombin activity may prolong the PT. Retesting the sample using a different PT reagent and performing factor II assays can be diagnostically helpful (Tripodi et al. 2001; Mazodier et al. 2012). Alternative tests that are insensitive to the effect of lupus anticoagulants, such as a chromogenic factor X assay, may be used to monitor the warfarin anticoagulant effect in such cases (Moll and Ortel 1997; Kasthuri and Roubey 2007).

Activated Partial Thromboplastin Time (aPTT)

The aPTT assesses the intrinsic and common pathways (factors XII, XI, IX, VIII, X, V, II, and I) (Fig. 1.1). Patient plasma is incubated for a short time with a partial thromboplastin, which is a tissue factor-free source of phospholipid, and a negatively charged surface, such as kaolin or silica, which can activate the contact factors. The sample is then recalcified. The aPTT is the time (in seconds) that it takes to form a fibrin clot after adding the calcium.

aPTT reagents differ in their sensitivity to heparin, coagulation factor deficiencies, and lupus anticoagulants (Bowyer et al. 2011; Fritsma et al. 2012; Gouin-Thibaut et al. 2012). Two main methods of clot detection, mechanical and optical, are used in coagulation testing with no clear advantage to one method over the other (McCraw et al. 2010). In some cases it may be necessary to recheck questionable aPTT (or other coagulation test) results by retesting the sample by both methods. For example, occasionally, an otherwise unexplained result of an aPTT that appears to be prolonged beyond the limit of detection actually reflects a markedly shortened aPTT. This occurs when the initial testing is performed using an optical detection method. Because the sample had already clotted before the optical method, which relies on a change in light transmittance, set its baseline, no change in light transmittance occurred over time and the result was erroneously interpreted as an aPTT prolonged beyond the limit of detection. In this case, the true shortened aPTT can be revealed by using a mechanical clot detection method (Milos et al. 2010).

Thrombin Time (TT) and Reptilase Time (RT)

The TT measures the time (in seconds) that it takes to convert fibrinogen to fibrin after adding exogenous thrombin to the patient sample (Fig. 1.1). The RT also measures the time it takes to convert fibrinogen to fibrin but differs from the TT in that the activation is thrombin independent and relies instead on an enzyme derived from a snake's venom (Zehnder 2013). Both the TT and RT are prolonged by fibrinogen abnormalities such as hypofibrinogenemia and dysfibrinogenemia, whereas the TT is prolonged and the RT is normal in the presence of unfractionated heparin or a direct thrombin inhibitor (DTI). LMW heparin does not typically prolong the TT or the RT. Additionally, the TT may be prolonged by high concentrations of FDPs and shortened in the presence of plasma volume expanders such as dextran and hydroxyethyl starch (Cunningham 2008).

Mixing Studies

aPTT mixing studies are utilized more frequently than PT mixing studies but all mixing studies share a common principle: the patient sample is mixed with normal plasma at a ratio of 1:1 and

the test of interest is performed. If the prolonged test "corrects" into the normal reference range, the result suggests a deficiency of one or more coagulation factors in the patient plasma. If the prolonged test does not correct, the result suggests the presence of an inhibitor. Weak inhibitors, however, can be diluted by the mixing study resulting in an apparent "correction" of the test (Moore and Savidge 2006). Additionally, significant deficiencies of multiple coagulation factors may not be completely corrected by the addition of normal plasma in a 1:1 ratio.

Mixing studies may be assessed at immediate and delayed phases. The delayed or time- and temperature-dependent phase reflects a period of incubation at 37° Celsius for 1–2 h prior to test performance. An immediate acting inhibitor typically suggests the presence of heparin, a DTI, or a nonspecific inhibitor such as a lupus anticoagulant. Specific factor inhibitors (such as a factor VIII inhibitor) may be associated with complete or partial correction at the immediate phase and a marked inhibitor effect at the delayed or time-dependent phase (Verbruggen et al. 2009).

Coagulation Factor Assays

Coagulation factors can be assessed by antigenic/immunologic or functional tests. It is usually most appropriate to test a factor's functional activity first and perform subsequent antigenic/immunologic testing only if there is clinical suspicion of qualitative factor abnormalities, which are far less common than quantitative factor deficiencies. The functional activity of a factor is typically determined by its ability to correct the clotting time of a standard plasma deficient in only the factor of interest (Mackie et al. 2013).

Similar to the aPTT, clot-based factor assays are subject to interference by inhibitors such as heparin, DTIs, and lupus anticoagulants. Normally, serial dilution of a patient sample is expected to result in decreased levels of factor activity with each dilution; however, in the presence of an inhibitor, a dilutional effect is noted whereby the factor activity level increases, rather than decreases, with dilution because of dilution of the inhibitor effect (Mackie et al. 2013).

Bethesda Assay

Although most commonly used to evaluate the potency of factor VIII inhibitors, the Bethesda assay can be used to assess the magnitude of the effect of any specific factor inhibitor by measuring the ability of the patient's plasma to neutralize the factor of interest in normal plasma. By definition, one Bethesda unit (1 BU) is the quantity of inhibitor that neutralizes 50 % of the factor of interest in normal plasma. Since the Bethesda assay is clot based, it is subject to interference by lupus anticoagulants, heparin, and DTIs, the presence of which may overestimate the effect of the specific factor inhibitor under study (Verbruggen 2010; Kershaw and Favaloro 2012).

Preanalytic Variables and Other Test Considerations

Coagulation tests are particularly sensitive to the effects of preanalytic variables. Extra care should be taken when collecting samples through indwelling lines due to the risk of contamination with heparin or other medications (Preston et al. 2010; Mackie et al. 2013). Sample collection tubes should be completely filled to ensure an appropriate ratio of plasma to anticoagulant (Adcock et al. 1998; McCraw et al. 2010; Preston et al. 2010; Mackie et al. 2013). Polycythemic patients with a hematocrit >55 % require an adjustment of the amount of anticoagulant in the test tube to avoid obtaining spuriously prolonged clotting times given the relatively low plasma to anticoagulant ratio in this case (Marlar et al. 2006; Preston et al. 2010; Mackie et al. 2013). Other considerations include handling, transport and storage conditions, and temperatures. Thawing and mixing of frozen samples prior to testing should also be performed with caution (McCraw et al. 2010; Preston et al. 2010; Mackie et al. 2013). Severely hemolyzed, turbid, or lipemic samples may impact coagulation test results, particularly when optical clot detection methods are used (Laga et al. 2006; Preston et al. 2010; Mackie et al. 2013).

Effect of Anticoagulants on Coagulation Assays

Anticoagulants can significantly affect the results of coagulation assays. Some of these effects are consistent and may aid in therapeutic drug monitoring; others merely complicate the laboratory evaluation of the patient's underlying hemostatic function. The effect of warfarin, heparin, and DTI on coagulation assays is relatively well established, whereas the effect of several new anticoagulants is still being evaluated (Favaloro et al. 2011).

The PT and INR are the first to prolong with oral vitamin K antagonist therapy (Ageno et al. 2012); prolongation of the aPTT is also typically observed in established warfarin therapy, particularly in case of INR greater than 1.5. In this setting, prolonged PTs and aPTTs are expected to correct fully on mixing studies. Warfarin therapy does not affect the thrombin time (TT) and is not expected to affect coagulation factor assays for procoagulant factors other than those which are vitamin K dependent (factors II, VII, IX, and X).

Unfractionated heparin exerts its primary anticoagulant effect by potentiating antithrombin (AT) (Garcia et al. 2012). The presence of unfractionated heparin would therefore be expected to prolong all clot-based assays due to its impact on the common pathway. The aPTT and TT are indeed prolonged in the presence of unfractionated heparin; however, in clinical laboratory practice, the PT appears to be unaffected by unfractionated heparin. This is because PT reagents contain a heparin-neutralizing agent that eliminates the heparin effect in nearly all cases. Valid results by other clot-based assays may also be obtained after treatment of the sample with a heparin-neutralizing agent, such as an enzyme or absorbing resin, as long as the heparin concentration in the sample does not exceed the neutralizing capability of these agents.

LMW heparins typically exert their primary anticoagulant effect through inhibition of FXa. The aPTT is variably prolonged in the presence of LMW heparins, whereas the PT and TT are typically unaffected by its presence. Heparin-neutralizing agents tend to be more effective at neutralizing unfractionated heparin than they are at neutralizing LMWH, both in vivo and in vitro (Garcia et al. 2012).

Oral and parenteral DTIs are currently available (Ageno et al. 2012; Garcia et al. 2012). DTIs act on thrombin and are associated with marked prolongation of the TT and variable prolongation of the PT and aPTT. DTIs have also been reported to interfere with several other coagulation assays, including mixing studies, factor assays, Bethesda assays, and tests for lupus anticoagulant. At the present time, no in vivo or in vitro DTI-neutralizing agents have been identified (Dager et al. 2012; Halbmayer et al. 2012; Adcock et al. 2013).

General Approach for Evaluation of Prolonged PT and/or aPTT Results

Prolongations of the PT and aPTT tend to garner the most amount of attention in clinical practice. Table 1.1 illustrates a broad approach to the differential diagnosis of various combinations of normal and prolonged PT and aPTT test results.

Shortened PT and aPTTs are evaluated less frequently than prolonged ones. In most cases, shortened PTs and aPTTs are noted in clinical settings physiologically associated with increased coagulation factor levels, such as pregnancy and acute phase reactions. Shortened PTs and/or aPTTs may also be seen in some cases of dysfibrinogenemia. A shortened aPTT is not currently considered to be a definitive indicator of hypercoagulability, but both shortened aPTTs and increased levels of some coagulation factors, including FVIII and FIX, which can cause a shortened aPTT, have been associated with an increased risk of thrombosis (Tripodi et al. 2004; Jenkins et al. 2012).

Laboratory Assays for Evaluation of Hypercoagulability

Hypercoagulable status, also called *thrombophilia*, is described as a group of hereditary or acquired conditions with the propensity to

Table 1.1 General approach to the differential diagnosis of combinations of normal and prolonged PT and aPTT results

PT	aPTT	aPTT mixing study	Diagnostic possibilities
Normal	Normal	Not indicated	Normal
			Coagulation abnormalities not assessed by the PT and aPTT (e.g., factor XIII deficiency)
Prolonged	Normal	Not indicated	Factor VII deficiency
			Mild deficiencies of common pathway factors (X, V, II, fibrinogen)
			Early warfarin/vitamin K deficiency effect, early liver disease, early disseminated intravascular coagulation (DIC)
Prolonged	Prolonged	Corrects	Congenital deficiency of a single coagulation factor in the common pathway, or combined deficiencies of factors in the common and/or intrinsic and extrinsic pathways
			Acquired deficiency of a single factor in the common pathway (e.g., factor X deficiency in amyloidosis)
			Acquired deficiencies of multiple coagulation factors (e.g., established warfarin/vitamin K deficiency effect, established liver disease, consumptive coagulopathy such as DIC, dilutional coagulopathy)
			Dysfibrinogenemia
			Weak inhibitor effect that is diluted out
		Does not correct	Inhibitors: inhibitor of a specific coagulation factor in the common pathway (e.g., factor V inhibitor); direct thrombin inhibitor (DTI); lupus anticoagulant (rarely associated with prolonged PT); high heparin (very rarely associated with prolonged PT) or combined heparin and warfarin therapy
			Profound deficiency of multiple coagulation factors that is not completely corrected by mixing with normal plasma
Normal	Prolonged	Corrects	Deficiencies of one or more of the intrinsic pathway factors (XII, XI, IX, and VIII) or the contact factors (prekallikrein and high-molecular-weight kininogen)
			Weak inhibitor effect that is diluted out
		Does not correct	Non-specific inhibitors: lupus anticoagulants; heparin; DTI
			An inhibitor of a specific factor in the intrinsic pathway (e.g., factor VIII inhibitor)

Table 1.2 Summary of risk factors for acquired and hereditary thrombophilia

Acquired thrombotic risk factors	Hereditary thrombotic risk factors
Major surgery/trauma	Activated protein C resistance/factor V Leiden
Immobilization	Prothrombin gene G20210A mutation
Solid or hematologic malignancies (e.g., myeloproliferative neoplasm)	Protein C deficiency[a]
Pregnancy	Protein S deficiency[a]
Oral contraceptives	Antithrombin deficiency[a]
Estrogen replacement therapy	Hyperhomocysteinemia[a]
Limb immobilization (e.g., hip/knee replacement, prolonged cast, stroke)	Elevated factor VIII activity[a]
Bedridden due to illness	Dysfibrinogenemia[a]
Antiphospholipid antibody syndrome	
Heparin-induced thrombocytopenia	
Paroxysmal nocturnal hemoglobinuria	
Obesity	
Nephrotic syndrome	
Smoking	

[a]Can be hereditary or acquired

develop venous thrombosis, arterial thrombosis, or both. Although acquired or hereditary thrombotic risk factors are not completely understood, the prevalence of factor V Leiden mutation, prothrombin gene G20210A mutation, elevated factor VIII, and hyperhomocysteinemia is higher than antithrombin, protein C, or protein S deficiencies in general population. The prevalence of thrombosis in individuals with a personal and/or family history of thrombosis is higher than in the general population. Acquired and hereditary risk factors for thrombophilia are summarized in Table 1.2 (Eby and Olson 2008a; Margetic 2010). Although patients with hypercoagulable risk factors are at a great risk for developing a thrombotic event, not all patients with hypercoagulable risk factors will develop an overt thrombosis and not all patients with thrombosis will have an identifiable hypercoagulable state (Kottke-Marchant 1994; Khor and Van Cott 2009; Margetic 2010).

Diagnostic thrombophilia testing is indicated in patients with idiopathic or recurrent venous thromboembolism (VTE), a first episode of VTE at a young age (<40 years), VTE in the setting of a strong family history, VTE in an unusual vascular site (cerebral, hepatic, mesenteric, or renal veins), neonatal purpura fulminans, warfarin-induced skin necrosis, and recurrent pregnancy loss (Heit 2007; Baglin et al. 2010). No single laboratory test is available to identify all hypercoagulable defects. Selection for thrombophilia testing differs depending on location and type of thrombosis. Many tests currently used to detect thrombophilia can be often affected by concurrent clinical conditions. In selected patients with thrombophilia, it is best to test for all recognized hereditary risk factors, both common and uncommon (Heit 2007; Eby and Olson 2008a; Middeldorp 2011). Figure 1.3 outlines a testing algorithm to maximize diagnostic potential in patients with thrombophilia. Testing should be performed in a stepwise manner beginning with high-yield screening tests followed by appropriate specific confirmatory tests. These comprehensive panels generate multiple test results which can each be affected by a variety of clinical conditions and drugs. Comprehensive narrative interpretation by coagulation specialists is necessary to synthesize the test results and correctly interpret them in the clinical context (Eby 2008b; Margetic 2010).

Appropriate timing for diagnostic thrombophilia testing is of critical importance. Tests should be performed at least 4–6 weeks after an acute thrombotic event or discontinuation of anticoagulant therapies including warfarin, heparin, DTIs, and fibrinolytic agents (Eby and Olson

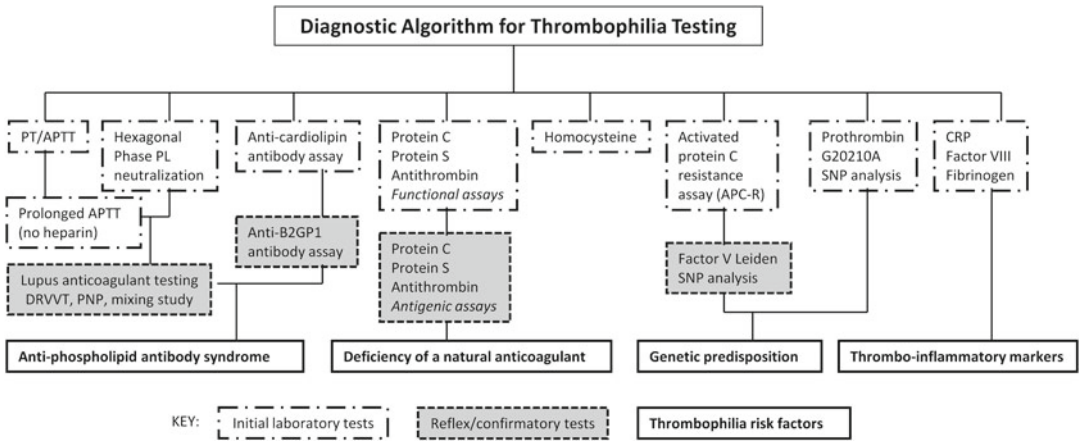

Fig. 1.3 Comprehensive diagnostic interpretive panel of laboratory tests for thrombophilia (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2013. All Rights Reserved). *aPTT* activated partial thromboplastin time, *B2GP1* β2 glycoprotein 1, *CRP* C-reactive protein, *DRVVT* dilute Russell's viper venom test, *PL* phospholipid, *PNP* platelet neutralization procedure, *PT* prothrombin time, *SNP* single nucleotide polymorphism

2008a; Heit 2007; Khor and Van Cott 2009). Acute thrombosis can cause elevation of acute phase reactants including factor VIII, C-reactive protein, beta chain of C4b-binding protein (C4bBP), fibrinogen, and IgM anticardiolipin antibodies. Warfarin therapy can lower protein C and protein S activity levels. Unfractionated or LMW heparins can affect antithrombin and lupus anticoagulant assays in addition to aPTT- and clot-based assays. Protein C, protein S, and antithrombin levels can be affected by liver dysfunction. Combined deficiency of protein C, protein S, and antithrombin can be observed in a consumptive coagulopathy, disseminated intravascular coagulation (DIC), liver disease, after recent thrombotic event, during the postoperative state, or with implantation of cardiovascular devices such as ventricular assist devices. Information about clinical conditions such as liver disease, pregnancy, or systemic inflammation should be provided to the laboratory to assure accurate test interpretation. Abnormal results should be repeated in a new specimen when the patient is in stable health and after anticoagulant therapy is discontinued. Alternatively, thrombophilia testing may be delayed until those clinical conditions have subsided. The one exception is DNA analysis for genetic mutations, which is not affected by anticoagulant therapy.

Activated Protein C Resistance and the Factor V Leiden Mutation

Activated protein C (APC) degrades activated coagulation factors Va and VIIIa in the presence of its cofactor protein S. APC resistance (APC-R) is observed in approximately 20 % of patients with first episode of deep vein thrombosis (DVT) and 50 % of familial thrombosis. Greater than 90 % of APC-R patients have a point mutation in the factor V gene, known as factor V Leiden (FVL) mutation (Rosendaal et al. 1995; Zivelin et al. 1997; Margetic 2010). FVL mutation causes a DNA polymorphism (G1691A) substituting amino acid arginine to glutamine at position 506 (R506Q), one of the three arginine sites (R306, R506, and R679) cleaved by APC (Eby and Olson 2008a). FVL is present in heterozygous form in approximately 3–5 % of the general Caucasian population and is rare in African, Australian, or South Asian populations (Ricker et al. 1997; Margetic 2010). The FVL mutation is the most common known hereditary risk factor for venous thrombosis. However, its risk in arterial thrombosis is not yet clear. The risk of venous thrombosis is increased four- to eightfold in individuals heterozygous for FVL and 80-fold in homozygotes (Greengard et al. 1994; Baglin et al. 2010). This thrombotic risk is further

increased in the presence of a second risk factor. Women with FVL mutation (heterozygous) using oral contraceptives appear to have a 30- to 60-fold increased risk of thrombosis. Some studies show that the risk of pulmonary embolism (PE) is not as great as the risk of DVT in FVL mutation patients (Rosendaal et al. 1995). In addition to FVL mutation, various less common FV mutations including FVR2 haplotype (H1299R), FV Liverpool (I359T), FV Cambridge (R306T), and FV Hong Kong (R306G) also affect APC resistance and thrombotic risk (Chan et al. 1998; Franco et al. 1999; Norstrom et al. 2002).

Acquired APC-R can be caused by development of autoantibodies against factor V following exposure to bovine thrombin, or with untreated hematological malignancies, lupus anticoagulants, pregnancy, oral contraceptives, active thrombosis, elevated factor VIII, and mutations in the factor VIII gene (Khor and Van Cott 2009; Margetic 2010).

Laboratory assays for APC-R and FVL mutation include functional assays and genotyping for FVL by DNA analysis. The functional assay for APC-R is based on prolongation of aPTT by degradation of factors Va and VIIIa by exogenously supplied APC. The ratio of aPTT in patient plasma and normal plasma before and after adding APC is calculated. The ratio in normal individuals is 2.0 or higher; heterozygous individuals for FVL mutation will have ratio of 1.5–2.0, and homozygous individuals will have ratio of less than 1.5. Each laboratory should determine its own cutoff for an abnormal result (Khor and Van Cott 2009; Yohe and Olson 2012). Elevated factor VIII, low protein S (less than 20 %), and causes of prolonged baseline aPTT such as heparin, warfarin, DTI, lupus anticoagulant, liver dysfunction, or low factor levels can cause a falsely low APC-R ratio. A second generation assay for functional APC-R uses pre-dilution of patient plasma with factor V-deficient plasma (also containing heparin neutralizer) and provides higher sensitivity and specificity. This assay is less affected by active thrombosis, surgery, inflammatory condition, heparin, or warfarin (Press et al. 2002). Identification of FVL mutation as the cause of APC-R is confirmed by DNA analysis such as polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) or allele-specific PCR (AS-PCR) genotyping. Non-PCR-dependent and simple microtiter plate-based Invader technology using fluorescence resonance energy transfer (FRET) mechanism shows a reliable detection rate for FVL mutation. However, due to the use of specific primers, this test method will only detect specific mutations, i.e., FVL mutation, and will not detect other rare FV mutations related to functional APC-R. In general, a cost-efficient functional assay for APC-R is recommended as an initial screening test, with DNA analysis for FVL mutation in individuals with abnormal APC-R results (Ledford et al. 2000; Murugesan et al. 2012).

Prothrombin Gene G20210A Mutation

The prothrombin gene mutation is a gain of function mutation which results in elevated functional prothrombin (factor II) level due to increased synthesis. A mutation which changes guanine to adenine at the 20210 position of the PT gene (G20210A) occurs in an intron near the 3′ end of the gene. This alters mRNA formation by affecting 3′ end processing and/or enhancing translation efficiency, resulting in increased plasma protein levels (McGlennen and Key 2002). However, the exact mechanism of how increased prothrombin gene expression causes hypercoagulability remains unclear. The prothrombin gene G20210A mutation is the second most common hereditary risk factor for venous thrombosis (Eby and Olson 2008a; Margetic 2010). Prevalence varies by ethnic group; 2–4 % of Europeans carry the mutation, which is rare in Asians, native Americans, or Africans (Rosendaal et al. 1998; Ballard and Marques 2012; Yohe and Olson 2012). This mutation is present in approximately 1–3 % of the general population, 5–10 % of patients with venous thrombosis, and up to 20 % of patients with venous thrombosis from thrombophilic families. Heterozygous individuals show threefold increased risk of venous thrombosis. However, venous thrombosis risk will be

drastically increased when the patients carry additional inherited or acquired risk factors (Poort et al. 1996; Khor and Van Cott 2009; Margetic 2010).

The prothrombin gene testing to detect the 20210 single nucleotide polymorphism (SNP) can be performed by various PCR-based methods. A commonly utilized method is PCR followed by DNA sequencing by gel electrophoresis, restriction endonuclease digestion, and radioisotope probing. Technologic advances in molecular diagnostic testing have led to automated genotyping analyses based on various PCR methods coupled with fluorescence polarization methods or the Invader assay. DNA microarray technology can detect multiple genetic markers simultaneously using various DNA chip platforms with a relatively low cost as a single test compared to conventional DNA assays (McGlennen et al. 1992; Murugesan et al. 2012). The DNA analysis cannot be affected by other conditions such as active thrombosis, surgery, inflammatory conditions, or anticoagulant therapy. However, these tests require expensive equipment and skilled personnel, have the risk of contamination, and may require reflex confirmatory assays (e.g., sequencing) if there is an ambiguous or atypical pattern by PCR (Murugesan et al. 2012).

Protein C Deficiency

Protein C is a vitamin K-dependent glycoprotein primarily synthesized as an inactive form by the liver (Khor and Van Cott 2010a). Activation of functional protein C requires interaction with the thrombin–thrombomodulin–endothelial protein C receptor complex and cofactor protein S. Protein C regulates thrombin generation by degradation of activated coagulation factors Va and VIIIa (Eby and Olson 2008a). Protein C deficiency occurs in 0.14–0.50 % of the general population and an estimated 1–3 % of patients with VTE (Khor and Van Cott 2009; Yohe and Olson 2012). It is inherited in an autosomal dominant fashion. Risk for venous thrombosis increases sevenfold in heterozygotes (Ballard and Marques 2012). Such individuals usually shows functional protein C levels of 40–65 % of normal. The first thrombotic event usually presents between the ages of 10 and 50 years (Khor and Van Cott 2009). Protein C deficiency also carries increased risk for warfarin-induced skin necrosis. Patients who are homozygous for the deficiency are very rare and can present with neonatal purpura fulminans or disseminated intravascular coagulation (DIC).

Protein C assays measure either protein C activity (functional) or antigen quantity (immunological). As an initial test, functional protein C assay, which provides a measure of both functional and antigenic levels, is commonly performed. If the result is low, an antigenic protein C assay is required. Type 1 protein C deficiency is quantitative and characterized by reduced functional activity and antigen levels and more common than type 2 deficiency, which is a qualitative defect, resulting in reduced activity and normal antigen level. If only a quantitative antigenic assay is used, type 2 deficiency cannot be detected (Eby and Olson 2008a; Margetic 2010). Functional protein C levels can be measured by clotting time-based or chromogenic assays. Clotting time-based assay can detect both type I and type 2 deficiency. However, it can give falsely increased results with anticoagulant therapy, lupus anticoagulant, and FVL mutation and falsely decreased results with elevated factor VIII levels (particularly greater than 250 %) or low protein S (in acute phase response). The chromogenic assay is less affected by interfering substances than the clotting time-based assay and is more reproducible; however, it can detect only functional protein C related to the peptide-binding site and therefore can miss some type 2 deficiencies (Eby 2008b; Khor and Van Cott 2009; Yohe and Olson 2012).

Acquired protein C deficiency is more common than hereditary protein C deficiency. The acquired causes should be excluded first before making a diagnosis of hereditary protein C deficiency. Because protein C is synthesized in hepatocytes and is vitamin K dependent, both liver dysfunction and vitamin K deficiency (including warfarin therapy) can decrease protein C levels. Protein C has a short half-life (6–8 h), and protein

C levels are more rapidly reduced in liver disease, anticoagulation therapy, and vitamin K deficiency compared to other coagulation proteins such as protein S or antithrombin. Conversely protein C levels rapidly recover into the normal reference range after discontinuation of anticoagulation therapy or correction of vitamin K deficiency. Even so, to ensure the most accurate results, protein C levels should not be measured for at least 10 days after stopping anticoagulants such as warfarin. Protein C levels are lowered in recent or current thrombosis, DIC, l-asparaginase therapy, and nephrotic syndrome and during the intra- or immediately postoperative period; neonates also have relatively low protein C levels (range 17–53 %). Oral contraceptive use and pregnancy can increase protein C level (Van Cott et al. 2002; Eby 2008b; Margetic 2010). Initially low protein C assays should be repeated after any such conditions have resolved.

Protein S Deficiency

Protein S is a vitamin K-dependent glycoprotein which acts as a cofactor to protein C, accelerating protein C proteolysis of factor Va and VIIIa approximately twofold (Maurrissen et al. 2008). Approximately 60 % of protein S in the plasma is bound non-covalently to C4bBP in plasma with high affinity; the remaining free (unbound) protein S is the predominantly active form. Recent studies have shown that protein S also exerts its own anticoagulant activity by direct binding of factors V, VIII, and X and also appears to act as a cofactor for the tissue factor pathway inhibitor, which results in inhibiting tissue factor-mediated factor X activation (Maurrissen et al. 2008; Rosing et al. 2008). Hereditary protein S deficiency is transmitted in an autosomal dominant fashion. Protein S deficiency occurs in 0.2–0.5 % in general population and 1–3 % of patients with first venous thrombosis (Dykes et al. 2001; Eby and Olson 2008a; Khor and Van Cott 2010a). Functional protein S levels range between 20 and 64 % in heterozygous patients (Aillaud et al. 1996). Homozygous patients typically present as newborns with purpura fulminans and DIC.

There are three types of protein S deficiency. Type I and type III are quantitative deficiencies with both low free protein S antigen and low protein S activity and together account for 95 % of cases. Type I deficiency can be further differentiated from type III deficiency as the former shows low total protein S antigen level while the latter shows normal total protein S levels. Type III deficiency may be related to excess binding of protein S to C4bBP. Type II deficiency is a qualitative defect with low protein S activity with normal antigenic free and total protein S levels (Castodi and Hackeng 2008; Ten Kate et al. 2008; Ballard and Marques 2012).

There are three protein S assays measuring its activity (functional) and antigen levels of free and total protein S (immunological). As with protein C, the functional activity assay, which can detect quantitative and qualitative protein S deficiencies, is the recommended initial test, and the antigenic assay is performed only if functional activity is low. Functional protein S activity measured by clotting time-based assay is sensitive, but not specific. Measurement of antigenic protein S is currently performed by monoclonal antibody-based enzyme immunoassay and immunoturbidimetric assay.

Acquired causes of protein S deficiency should be excluded before making a diagnosis of protein S deficiency. Protein S will be decreased in clinical conditions which decrease protein C (see above). In addition to those conditions, protein S will be also decreased in acute phase response because its binding protein C4bBP is an acute phase reactant; when C4bBP is increased, it lowers both protein S activity and free antigen. Protein S is also decreased with elevated factor VIII (greater than 250 %) and infectious and autoimmune conditions such as HIV infection, Crohn's disease, or ulcerative colitis (Dykes et al. 2001; Khor and Van Cott 2009; Yohe and Olson 2012). Protein S levels are usually lower in women, especially during hormone replacement therapy, oral contraceptive use, and the second or third trimester of pregnancy (Eby and Olson 2008a). Protein S level should be repeated after any such conditions causing acquired protein S deficiency are resolved.

Antithrombin Deficiency

Antithrombin is a glycoprotein of the serine protease inhibitor (serpin) family which primarily inactivates activated thrombin (factor IIa) and factor Xa and, to a lesser extent, factors IXa, XIa, and XIIa. Antithrombin acts as a so-called suicide inhibitor by forming a 1:1 covalent complex between antithrombin and serine proteases. This inhibitor activity is greatly accelerated by interaction with heparin. Although antithrombin is synthesized in the liver parenchyma, it is not vitamin K dependent and has a half-life of 2–3 days (Vossen et al. 2004; Rogers and Kottke-Marchant 2012).

Antithrombin deficiency is inherited in autosomal dominant fashion. The prevalence rates are approximately 0.05–0.1 % in the general population. Estimated annual incidence of a first episode of VTE in carriers of antithrombin deficiency is 1.0–2.9 % per year in retrospective studies (Yohe and Olson 2012; Khor and Van Cott 2010b). Antithrombin deficiency is associated almost exclusively with venous thrombosis. In general, the risk of thrombosis appears to be higher in antithrombin deficiency than for protein C or protein S deficiency, APC-R, or prothrombin gene G20210A mutation and thus has the highest risk for VTE among the known hereditary thrombophilias. Most cases are heterozygous because homozygosity for antithrombin deficiency is almost universally fatal in utero. Functional antithrombin levels in heterozygous individual range from 35 to 70 % (Kottke-Marchant and Duncan 2002; Picard et al. 2000; Khor and Van Cott 2009; Middeldorp 2011). VTE in antithrombin deficiency usually occurs as DVT of the extremities and PE but can also occur in unusual sites, such as cerebral sinuses, mesenteric, portal, and renal veins. It usually occurs at a young age (<50 years), but it is uncommon during the first two decades of life and may or may not follow a provocative event. Approximately 58 % of these episodes occur spontaneously, while 42 % show an association with a transient risk factor, which can be potentially preventable. Patients with concurrent defects such as factor V Leiden mutation are associated with higher risk of VTE and at younger ages with a median age of 16 years (Dykes et al. 2001; Khor and Van Cott 2010b).

Both antithrombin functional activity and antigen quantity can be measured. Assays of antithrombin function are predominantly chromogenic assays. If an initial functional level is normal or elevated, antithrombin deficiency is unlikely. If it is low, then a confirmatory functional test should be done on the patient using a repeat specimen. On the repeat specimen, both functional and antigenic levels are tested to determine whether the patient has a type 1 or type 2 antithrombin deficiency. Antigen levels can be measured by enzyme immunoassays and immunoturbidimetric methods (Khor and Van Cott 2009; Yohe and Olson 2012).

There are two major types of inherited antithrombin deficiency. Type 1 antithrombin deficiency is most commonly caused by a lack of the antithrombin gene product showing proportionately reduced functional and antigenic levels. It is frequently observed in the heterozygote state, resulting in approximately 50 % activity and antigen levels. Type 2 antithrombin deficiency is a qualitative deficiency, resulting in lower functional activity than antigen levels. Type 2 antithrombin deficiencies are further classified by antithrombin mutation site and performance on different antithrombin assays: (1) type 2a mutations affect the antithrombin reactive site, (2) type 2b mutations cause abnormalities in the heparin-binding site, and (3) type 2c mutations have a pleiotropic effect affected on both sites. Type 2c pleiotropic defects are associated with a moderate decrease in both antithrombin function and antigen levels (typically function levels are lower than antigen levels). However, subclassification is generally not clinically necessary because anticoagulant therapy does not differ between types (Dykes et al. 2001; Vossen et al. 2004; Picard et al. 2000; Rogers and Kottke-Marchant 2012).

Acquired antithrombin deficiency must be excluded before making a diagnosis of hereditary antithrombin deficiency and can be caused by drugs such as heparin or L-asparaginase. Other causes of low antithrombin levels include reduced synthesis in liver disease and increased anti-

thrombin loss in nephrotic syndrome, protein-losing enteropathy, DIC, sepsis, burn, trauma, hepatic veno-occlusive disease, thrombotic microangiopathies, cardiopulmonary bypass surgery, hematomas, or metastatic tumors. Antithrombin activity can be reduced by up to 30 % during full-dose unfractionated heparin therapy, but will not be reduced during LMW heparin therapy, and levels will normalize when heparin is discontinued. Antithrombin levels can be low in premenopausal women, oral contraceptive use, and pregnancy. As with low protein C and low protein S, confirmatory testing should be repeated on a new specimen after any potential confounding conditions have resolved (Rao et al. 1981; Kottke-Marchant and Duncan 2002; Eby 2008b; Khor and Van Cott 2009).

Antiphospholipid Syndrome

Antiphospholipid syndrome (APS) is the most common cause of acquired thrombophilia. The presence of antiphospholipid antibody (APA) is associated with an increased risk of both arterial and venous thrombosis and recurrent pregnancy loss. APAs are acquired autoantibodies directed against phospholipid–protein complexes and are present in 3–5 % of the general population (Van Cott and Eby 2008; Miyakis et al. 2006). There are both primary and secondary forms of APAs arising spontaneously or in association with another condition. These antibodies, also known as lupus anticoagulants (LA) due to their prevalence in patients with systemic lupus erythematosus (SLE), are extremely heterogeneous and are directed against a wide variety of anionic phospholipids, including cardiolipin, beta2 glycoprotein 1 (B2GP1), or cell-membrane phosphatidylserine (Kottke-Marchant 1994; Pengo et al. 2009).

Diagnosis of APS is made by clinicopathologic evaluation. In addition to clinical criteria such as vascular thrombosis or pregnancy morbidity, repeated laboratory testing of APA is required for diagnosis because transient low level increases in APA can be detected in a variety of clinical conditions including acute phase response. Laboratory diagnostic criteria include positive testing for one of the following on two or more occasions, at least 12 weeks apart: (1) lupus anticoagulant, (2) anticardiolipin antibodies (IgG or IgM) in medium or high titer, and (3) B2GP1 antibodies (IgG or IgM) in medium or high titer (Finazzi et al. 1996; Miyakis et al. 2006; Heit 2007; Pengo et al. 2009). Based upon consensus criteria from the International Society for Thrombosis and Haemostasis (ISTH), confirmation of LA requires that the following four criteria should be met (Brandt et al. 1995; Pengo et al. 2009). First, there should be prolongation of at least one phospholipid-dependent clotting test (e.g., aPTT, dilute Russell's viper venom test [DRVVT] screen, or hexagonal phospholipid neutralization screen). DRVVT is considered as the screening test, and the second test should be a sensitive aPTT with low phospholipids and silica as an activator. Second, there is an evidence of inhibitory activity in the patient plasma demonstrated by mixing patient plasma with pooled normal plasma (e.g., immediate and incubated mixing study or DRVVT mixing study). Third, phospholipid dependence of the inhibitor should be demonstrated on a confirmatory test which demonstrates shortening of clotting time with the addition of more phospholipid (e.g., DRVVT confirmatory ratio, hexagonal phospholipid neutralization ratio, platelet neutralization). Fourth, the presence of a specific factor inhibitor, particularly factor VIII, and anticoagulant drugs such as heparin or direct thrombin inhibitor should be excluded (Brandt et al. 1995; Reber and de Moerloose 2004; Miyakis et al. 2006; Pengo et al. 2009; Nichols et al. 2012).

Paradoxically, LAs prolong clot-based assays in vitro while predisposing to thrombosis in vivo. In fact, approximately 30 % of LA patients will experience thrombosis. In approximately 15 % of patients with DVT, clotting is attributable to the presence of LA (Margetic 2010; Yohe and Olson 2012). Because no single test is available to detect LA, laboratory testing for LA consists of a panel of assays following a diagnostic algorithm (Fig. 1.4). To maximize diagnostic potential, at least two assays based on different principles should be performed to fulfill each of the four criteria, and assays are usually performed with low

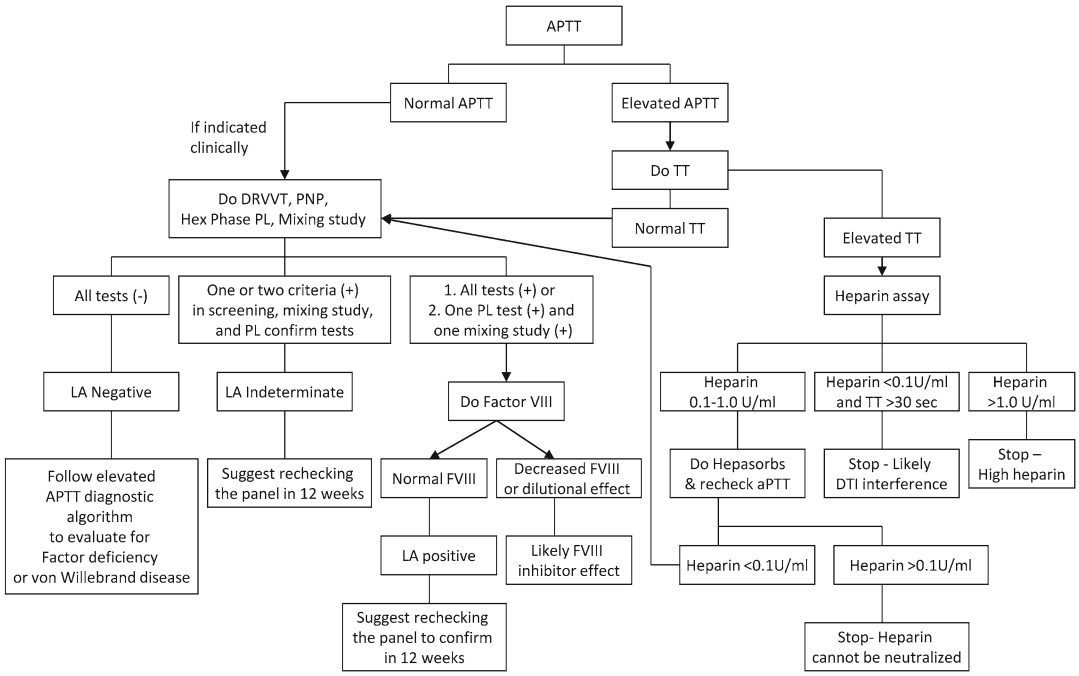

Fig. 1.4 Diagnostic algorithm for detection of lupus anticoagulant (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2013. All Rights Reserved). *aPTT* activated partial thromboplastin time, *DRVVT* dilute Russell's viper venom test, *FVIII* factor VIII, *Hex* hexagonal, *LA* lupus anticoagulant, *PL* phospholipid, *PNP* platelet neutralization procedure, *TT* thrombin time

concentrations of phospholipid to improve sensitivity. Tests for LA are interpreted as positive if the panel demonstrates one positive screening test, one positive mixing test, one positive confirmatory test, and no evidence for a factor inhibitor or anticoagulant drug effect. If fewer than four diagnostic criteria are met and if clinical suspicion for LA exists, the test panel is interpreted as indeterminate and the patient should be retested at a later date (Miyakis et al. 2006; Van Cott and Eby 2008; Moffat et al. 2009; Pengo et al. 2009).

Updated guidelines for LA detection emphasize patient selection to minimize inappropriate requests of LA testing and avoid the risk of obtaining false-positive results. Level of appropriateness of LA testing is divided into three grades according to clinical characteristics. The low grade includes venous or arterial thromboembolism in elderly patients. Moderate grade includes prolonged aPTT in asymptomatic patients, recurrent spontaneous early pregnancy loss, and provoked VTE in young patients. High grade includes unprovoked VTE and arterial thrombosis in young patients (<50 years old), thrombosis at unusual sites, late pregnancy loss, and any thrombosis or pregnancy morbidity in patients with autoimmune disease. General searches for LA in asymptomatic individuals or patients other than those described here are highly discouraged (Pengo et al. 2009).

Specific antibodies against cardiolipin and B2GP1 (IgG or IgM) are measured by commercially available solid-phase enzyme-linked immunosorbent assay (ELISA). Anticardiolipin antibodies recognize a complex of cardiolipin, a naturally occurring phospholipid, bound to B2GP1 protein. Complexes of anionic phospholipids and endogenous plasma proteins provide more than one epitope recognized by natural autoantibodies. Detection of anticardiolipin antibodies is generally considered to be a sensitive test. However, because the antigen target of anticardiolipin antibodies is a B2GP1-cardiolipin complex, B2GP1 antibody assays are considered to be more specific than

anticardiolipin antibody assays (Triplett 2002; Galli et al. 2003; Marai et al. 2003). False-positive results for anticardiolipin antibodies can be associated with high level of rheumatoid factor and cryoglobulins (Finazzi et al. 1996; Miyakis et al. 2006).

Both anticardiolipin antibodies and B2GP1 APA assays are recommended because using just a B2GP1 antibody assay can miss some cases of APA. If a test result for APA diagnosis is positive, repeating the test in a new specimen after an interval of at least 12 weeks should be performed to confirm APA because transient occurrence of APAs can be caused by infection or drugs and is not associated with thrombotic risk (Margetic 2010; Van Cott and Eby 2008). In individuals with high-titer IgG anticardiolipin antibodies (>40 IgG phospholipid units [GPL]), a prospective study found a rate of thrombosis of 6.1 % per year, compared with 0.95 % per year in individuals with no history of thrombosis, 4.3 % in patients with SLE, and 5.5 % in patients with a history of thrombosis (Finazzi et al. 1996; Van Cott and Eby 2008; Khor and Van Cott 2009).

Because APAs have heterogeneous patterns of antigen recognition and different reagents vary in phospholipid composition, there are significant issues of preanalytic interference and interlaboratory variability which need to be considered for selection of APA assays and interpretation (Miyakis et al. 2006; Van Cott and Eby 2008; Pengo et al. 2009; Nichols et al. 2012). Frozen and thawed platelets, which can cause false-negative screening or mixing study results, should not be used. Pooled normal plasma should have a residual platelet count of less than 10,000/μL. Acute thrombotic events or acute phase responses with elevated factor VIII can cause false-negative results. Anticoagulant therapy, such as heparin or direct thrombin inhibitors, or presence of specific coagulation factor inhibitors can cause false-positive results with prolonged aPTT. Thrombin time can help to identify anticoagulant effect or specific inhibitors. Commercial reagents for LA testing include heparin neutralizers which can quench heparin concentrations up to 1.0 U/mL. However, similar reagents are not available for direct thrombin inhibitors; therefore, LA testing should not be performed on individuals taking these drugs. Individuals on long-term vitamin K antagonists should be tested 1–2 weeks after discontinuation of therapy after the INR has normalized to less than 1.5.

Hyperhomocysteinemia

Homocysteine is an intermediate amino acid produced by demethylation of methionine via methylenetetrahydrofolate reductase (MTHFR) in the folate cycle. The metabolism of homocysteine requires vitamin B6, vitamin B12, and folate. Hyperhomocysteinemia is associated with increased risk of arterial and venous thrombophilia and atherosclerosis. Acquired hyperhomocysteinemia can be caused by deficiency of vitamin B6, vitamin B12 or folate, renal failure, hypothyroidism, psoriasis, inflammatory bowel disease, rheumatoid arthritis, and therapy with certain drugs such as methotrexate, niacin, anticonvulsants, theophylline, l-dopa, thiazide, cyclosporine A or phenytoin (Guba et al. 1999; Eldibany and Caprini 2007; Khor and Van Cott 2009).

Hereditary hyperhomocysteinemia is caused by a mutation in an enzyme in homocysteine conversion pathways. Homozygous mutations of the MTHFR gene are present in 10–13 % of the population, while heterozygous mutations are found in 30–40 % (Guba et al. 1999). A common mutation in the MTHFR gene is C677T, which is a polymorphism with a C to T substitution at nucleotide 677. This mutation has been known to be related to thrombosis risk; however, meta-analyses have found only a weak association (Ray et al. 2002; Deb Heijer et al. 2005; Eby 2008b). Homozygosity for MTHFR C677T is associated with approximately 25 % increase in total plasma homocysteine level. Hyperhomocysteinemia results in a three- to fivefold increase in the risk of coronary artery disease. Lowering homocysteine levels by therapy with vitamin B6, vitamin B12, or folate has not been proven to reduce thrombotic risk (Van Cott et al. 2002; Khor and Van Cott 2009). Given the modest risk of homocysteine on thrombophilic risk, variable prevalence in different ethnic groups (higher prevalence in Caucasian and lower prevalence in African-Americans), and lack of evidence of therapeutic benefit, screening

homocysteine levels in healthy individuals and testing for MTHFR mutations are not currently suggested.

Elevated Factor VIII

Several thrombophilia studies have demonstrated an association between elevated factor VIII and increased risk of thrombophilia, due at least in part to factor VIII-mediated enhancement of thrombin generation (O'Donnell et al. 2000; Oger et al. 2003; Ota et al. 2011; Jenkins et al. 2012). To date no genetic variations in the factor VIII gene have been identified. Factor VIII appears to be higher in African-Americans and lower in blood group O. The prevalence of elevated factor VIII among patients with venous thrombosis is 20–25 % (Kraaijenhagen et al. 2000; O'Donnell et al. 2000; Jenkins et al. 2012). Elevation of factor VIII appears to be persistent over months to years and is independent of acute phase response. It is not clear yet if factor VIII elevation directly contributes to increased thrombophilic risk. However, studies show that persistent factor VIII level greater than 150 %, or greater than the 90th percentile in the absence of acute phase reaction, elevated estrogen levels and recent exercise is an independent risk factor for thrombophilia (Kraaijenhagen et al. 2000; Kyrle et al. 2000; Benjaber et al. 2003).

Functional factor VIII activity can be measured by aPTT-based clotting assay or chromogenic assay, and antigen quantitation can be accomplished using ELISA. Factor VIII level can be elevated in acute phase reaction, elevated estrogen states, pregnancy, or after aerobic exercise. Factor VIII measurement should be postponed until at least 6 months after an acute thrombotic event and 6 weeks after giving birth and should be repeated after 3–6 months to confirm persistent elevation (Benjaber et al. 2003; Margetic 2010).

Fibrinogen Defects

Dysfibrinogenemia is a heterogeneous group of disorders resulting in structurally and functionally altered fibrinogen. It can cause bleeding, venous or arterial thrombosis, or both. The prevalence of dysfibrinogenemia in patients with venous thrombosis is approximately 0.8 % (Haverkate and Samama 1995; Cunningham et al. 2002). Although the mechanism of thrombosis is unknown, increased fibrin formation or impaired fibrinolysis may be associated with thrombosis. Dysfibrinogenemia patients can have prolonged prothrombin time, thrombin time and reptilase time, decreased functional fibrinogen, and normal to elevated immunologic fibrinogen. The ratio of functional fibrinogen activity to immunologic fibrinogen antigen will be decreased in dysfibrinogenemia (Hayes 2002; Eby 2008b; Verhovsek et al. 2008; Margetic 2010).

The most commonly used functional assay is the Clauss method. Acquired deficiency of fibrinogen can be caused by liver disease, consumptive states such as placental abruption or DIC, or fibrinolytic therapy. As fibrinogen is an acute phase reactant, the test should be delayed at least 6 months after acute thrombosis (Verhovsek et al. 2008; Yohe and Olson 2012).

Laboratory Assays for Evaluation of Platelet Function

Platelet Structure, Activation, and Clot Formation

Platelets are small (2 μm) anucleate cells produced in the bone marrow from the cytoplasm of megakaryocytes. They circulate in the peripheral blood for 7–10 days at a normal concentration between 150,000 and 400,000/μL (George 2000). Platelet cytoplasm is filled with alpha and dense granules, each of which contains specific factors necessary for platelet function. Dense granules contain adenosine triphosphate (ATP) and adenosine diphosphate (ADP), 5-hydroxytryptamine (5-HT), histamine, and cations (Ca^{2+}, Mg^{2+}). Alpha granules are more complex and contain proteoglycans (e.g., platelet factor 4), adhesive glycoproteins (e.g., von Willebrand factor [vWF]), coagulation factors (e.g., fibrinogen; factors V, VII, XI, XIII; protein S), cellular

mitogens (e.g., platelet-derived growth factor, vascular endothelial growth factor), protease inhibitors (e.g., plasminogen activator inhibitor-I), and other miscellaneous molecules (e.g., immunoglobulins, albumin) (Rendu and Brohard-Bohn 2001). Also vital to platelet function is the platelet cytoskeleton, which forms the internal architecture of the cell and is responsible for platelet conformational changes, and the cell membrane, upon which specific receptors, adhesion molecules, and antigens are anchored (Hartwig 2006).

Platelets respond to endothelial injury with formation of a hemostatic platelet plug. This occurs through a process of adhesion to the site of injury followed by activation, which includes degranulation, and potentiates the platelet for the next step, aggregation (platelet–platelet binding) (Jackson 2007). There are several redundant pathways and positive feedback loops in this process, which provide multiple targets for antiplatelet agents. Platelet activation and aggregation also has multiple synergistic interactions with the coagulation cascade. For example, the platelet alpha granules contain procoagulant molecules (fibrinogen, vWF, factor V) and molecules exposed on the surface of activated platelets provide sites for phospholipid-dependent coagulation complexes. When a platelet encounters the exposed subendothelium of a damaged blood vessel, extracellular matrix proteins interact with receptors on the platelet surface. These interactions lead to rapid platelet adhesion to the site of vascular injury. This initial platelet adhesion is via a weak bond ("rolling") between GPIb/IX/V on the platelet membrane and vWF secreted by endothelial cells. This binding also triggers the release of Ca^{2+} from internal platelet stores which function in platelet activation. Further binding occurs between exposed collagen and collagen receptors on the platelet surface. Binding of collagen to the GPVI/FCRγ complex stimulates platelet activation, while binding to the GPIa/IIa collagen receptor creates stable adhesion ("tethering") (Jackson 2007). In addition to vWF and collagen, there are multiple other triggers, or agonists, of platelet activation including thrombin, epinephrine, and ADP. Binding of ADP to the high-affinity $P2Y_1$ receptor is responsible for inducing platelet shape-changing, release of cytoplasmic Ca^{2+}, and the initial wave of aggregation. However, full aggregation cannot be triggered without binding to the low-affinity ADP receptor $P2Y_{12}$. Binding to this receptor also potentiates platelet secretion and thrombus stabilization. Prevention of binding to the $P2Y_{12}$ is the mechanism of action of thienopyridine drugs such as clopidogrel and prasugrel (Geiger et al. 1999).

Once the platelet is activated, it undergoes a change in shape, flattening out and increasing surface area. Platelet activation also leads to changes in cell surface molecules, including the fibrinogen receptor GPIIb/IIIa which is normally maintained in an inactive conformation on the cell surface. Once the platelet is activated, GPIIb/IIIa undergoes a conformational change which allows it to bind fibrinogen which in turn facilitates platelet aggregation via formation of interplatelet fibrinogen bridges (Jackson 2007). Thromboxane A_2 (TxA_2), a by-product of the platelet cyclooxygenase pathway, stimulates activation of adjacent platelets. Aggregation and platelet recruitment continues with eventual formation of the platelet plug. Fibrinogen binding to activated GPIIb/IIIa on the platelet surface also strengthens platelet adhesion to the site of endothelial injury. Because GPIIb/IIIa is so integral to platelet aggregation, antagonists of this molecule serve as potent antiplatelet agents (Harrington 1999), such as abciximab. Conversely aspirin exerts its antiplatelet function through irreversible inhibition of cyclooxygenase, preventing TxA_2 synthesis (Burch et al. 1978).

Preanalytic and Analytic Variables and Other Consideration in Platelet Testing

There are multiple preanalytic and analytic variables which can complicate laboratory assessment of platelets. Timing and specimen handling is of critical importance in platelet functional testing because platelets spontaneously activate in vitro. Blood should be drawn in a standardized fashion to minimize activation and aggregation. Blood should be collected in a tube with appropriate anticoagulant such as sodium citrate for platelet function testing and ethylenediaminetetraacetic acid (EDTA) for

platelet count. Hemolyzed, lipemic, and short draw specimens are not suitable for platelet function testing. Tests that assay platelet functional activity should be performed as soon as possible and certainly performed within 4 hours of collection. Specimens for function assays should be kept at room temperature; they cannot be chilled and rewarmed as this may cause loss of platelet function (Schmitz et al. 1998; Harrison 2004). Additionally specimens should not be sent via pneumatic tube (Dyszkiewicz-Korpanty et al. 2004; Hubner et al. 2010). These considerations necessitate careful coordination between the clinician and pathologist. Other measurements, such as platelet count, are more stable, and specimens can be tested up to 24 hours after phlebotomy (Gulati et al. 2002).

Drugs with antiplatelet activity are another common confounding variable in platelet testing and can lead to apparently abnormal platelet function, simulating an intrinsic platelet disorder. Drugs with antiplatelet activity include aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs), thienopyridines (clopidogrel, prasugrel, ticlopidine), dipyridamole, and glycoprotein (GP) IIb/IIIa inhibitors (abciximab, eptifibatide, tirofiban). As with any evaluation of bleeding or clotting issues, a thorough history, including all medications, is essential to proper platelet testing (George and Shattil 1991). If possible such drugs should be discontinued several days prior to when the specimen is drawn, and a listing of the drugs the patient is taking should be included with requests for platelet testing.

Laboratories should monitor assay performance by internal and external quality control programs to ensure consistent high levels of performance and accuracy (Dybiaer 1994; Hayward and Eilekboom 2007). However, complex platelet assays, such as aggregometry and flow cytometry, are not well standardized between laboratories compared to simple screening tests. Each laboratory may use different reagents, instrumentation, and standards (Moffat et al. 2005). Because of the evanescent nature of platelet function, controls for these tests cannot be stored or shipped and only a few tests have widely available clinical standards (Favaloro 2009). As such, it is important to realize that test results are often not directly comparable between laboratories.

Platelet Morphologic Assessment

Modern automated hematology instruments measure platelet number and size via impedance and/or modified flow cytometry. Additional platelet indices can be measured, analogous to those reported for erythrocytes, including mean platelet volume (MPV) and platelet distribution width (PDW). An increased MPV may be indicative of higher turnover as larger platelets are released from the bone marrow, and PDW may be elevated in myeloproliferative neoplasms (MPN) due to a mixture of giant and small platelets. The platelet count may be underestimated by automated systems in conditions with very large platelets, such as in congenital macrothrombocytopenias (e.g., Bernard–Soulier, MYH9) in which the platelets are artifactually counted as erythrocytes or lymphocytes. Some automated hematology analyzers are now able to measure immature platelet fractions, which can be used as a measure of platelet production (Ault et al. 1992).

Light microscopic examination of Wright- or Giemsa-stained peripheral blood smears can also be used to estimate platelet number and size. While visual examination is less precise than automated methods, microscopy can identify artifacts including artificially low platelet count due to platelet clumping (a common artifact in specimens collected in EDTA), satellitosis, or misidentification of giant platelets (Moreno and Menke 2002; Froom and Barak 2011). In addition, certain platelet disorders have characteristic morphologic anomalies; decreased or absent granularity is seen in alpha granule disorders such as gray platelet syndrome and occasionally MPN. True congenital macrothrombocytopenias can have uniformly giant platelets with very high MPV and normal PDW (Moreno and Menke, 2002; Mhawech and Saleem, 2000). Bone marrow examination can be useful in diagnosis of myelodysplastic syndromes, MPN, or other disease processes involving the marrow space.

Electron microscopy can be used to assess platelet shape and various properties of alpha and dense granules (Clauser and Cramer-Bordé 2009). However, EM is technically difficult, labor intensive, and costly and, as such, has mostly been supplanted by technologies such as flow cytometry.

Platelet Function Analysis

The classical screen of platelet function is the bleeding time (BT), determined by making a cut in the skin in the forearm, and observing time until cessation of bleeding (Harker and Slichter 1972). Results are influenced by multiple patient and operator variables including length, depth and site of incision, platelet number and function, skin temperature, fibrinogen concentration, and vascular function. The BT is poorly reproducible, unpopular with patients, and has not been shown to correlate with intraoperative bleeding (Lind 1991; Peterson et al. 1998). For these reasons, BT is no longer performed at most medical centers.

Platelet Function Screening

The platelet function analyzer (PFA)-100 system (Siemens Healthcare Diagnostics) is used by many centers as an in vitro alternative to BT to screen for global platelet function. The test assesses platelet function for both adhesion and aggregation at high shear rates, which mimics the properties of the human vasculature. Citrated blood is drawn through an aperture in a membrane coated with agonists (collagen/ADP and collagen/epinephrine). The time until occlusion of the aperture is recorded as the closure time (CT) up to 300 s; after that time CT is reported as >300 s (Kundu et al. 1995). The closure time is reflective of interactions of vWF with platelet membrane surface glycoproteins as well as platelet granularity and secretion and thus is prolonged in many cases of von Willebrand disease (vWD). Therefore, it can be used both as a screen of platelet function and vWD, but cannot differentiate between the two. The test may also have some utility in monitoring moderate vWD (Favaloro 2006). CT is sensitive to severe intrinsic platelet defects such as Glanzmann thrombasthenia and Bernard–Soulier syndrome but is less sensitive to disorders such as secretion defects and storage-pool disorders (Hayward et al. 2006). CT is not dependent on coagulation factor concentrations or heparin, but is affected by platelet count, hematocrit, and citrate concentrations. It is sensitive to GPIIb/IIIa inhibitors, and especially aspirin and NSAIDs, but cannot be used to monitor thienopyridines (Harrison 2005; Hayward et al. 2006). The aspirin effect is dose dependent and the test can be used to assay aspirin resistance (Crescente et al. 2008). Isolated abnormal patterns found using the collagen/epinephrine cartridge are often observed in specimens with aspirin-like drug effect or storage-pool disorders (Nurden and Nurden 2009; Kottke-Marchant et al. 1999). When used in conjunction with an adequate bleeding history, CT has proven to be predictive for bleeding risk. The PFA-100 is relatively simple and rapid (results in 5–8 min) and uses a small blood volume (800 μL) on a near-point-of-care platform. However, results are nonspecific and not sensitive for mild platelet dysfunction or vWD (Hayward et al. 2006).

Platelet Aggregation

Platelet aggregometry, which is considered the gold standard for platelet function testing, measures aggregation of platelets in a stirred sample in reaction to a variety of agonists. Different platelet disorders have different patterns of agonist response. Aggregometry can detect abnormalities in surface glycoproteins, signal transduction, and platelet granularity (Hayward et al. 2009). Optical platelet aggregometry is most commonly performed and is considered to be reflective of in vivo aggregation function of platelets. It uses platelet-rich plasma (PRP), processed by centrifugation of citrated blood, and measures changes (%) in light transmittance through the specimen with a modified spectrophotometer (turbidimetry). Aggregometry can also be performed on whole blood, in which case testing is based on changes in impedance (ohms) between two submerged probes as platelet aggregates form (Dyszkiewicz-Korpanty et al. 2005). The panel and concentration of agonists used varies by laboratories but usually includes ADP, collagen, arachidonic acid (AA), epinephrine, and occasionally thrombin receptor-activation peptide (TRAP) over a range of concentrations (Hayward et al. 2010). Classical platelet responses to agonists including lag, shape change, and primary and secondary aggregation are monitored and measured by maximal amplitude or percentage of aggregation after a fixed period of time (Nurden and Nurden 2009). Another important reagent used for platelet func-

tion is the antibiotic ristocetin which acts as a platelet agonist by facilitating binding of vWF to GPIb/IX/V by inducing the same activating conformational change in vWF as does high shear stress in vivo (Berndt et al. 1992).

Different platelet disorders show different patterns of aggregation in response to each of these agonists (Kottke-Marchant and Corcoran 2002). Characteristic aggregometric findings in different platelet disorders are summarized in Table 1.3. Ristocetin-induced platelet aggregation (RIPA) is measured at low and high concentrations and is sensitive to defects in some types of von Willebrand disease or GPIb/IX/V (Bernard–Soulier) (Jenkins et al. 1976). Patients with severe type 1 or type 3 vWD with markedly reduced vWF and type 2A with dysfunctional vWF can have a reduced response to ristocetin; however, patients with type 2B will have heightened response to lower concentration of ristocetin. Aggregometry alone is not very sensitive to storage-pool disorders, but sensitivity can be increased by the use of lumiaggregometry. This specialized test allows simult aneous measurement of platelet aggregation and ATP secretion measured as luminescence using the firefly luciferin–luciferase reaction (McGlasson and Fritsma 2009). Figure 1.5 is a diagram of normal lumiaggregation results showing simultaneous measurement of platelet aggregation and stimulated ATP release by dense granules during platelet aggregation. Aggregometry is a powerful tool for evaluating platelet disorders but is generally performed only in specialized centers. Aggregometry requires a large sample volume (~20 mL of whole blood) and takes several hours. Results are influenced by platelet count, which can be standardized somewhat with the use of PRP (Hayward et al. 2010). Centrifugation technique may alter test results and lipemic, hemolyzed, or icteric samples cannot be used for turbidimetric testing (Dyszkiewicz-Korpanty et al. 2005).

Platelet Flow Cytometry

Platelet flow cytometry is another powerful tool which can be used to evaluate multiple aspects of platelet structure and function. Flow cytometry simultaneously assesses multiple parameters of cells including size (forward scatter), granularity (side scatter), and presence of various molecules on the cell surface through the use of fluorescently labeled antibodies. Flow cytometry can be utilized to identify absence, decreased expression, or abnormalities of cell surface receptors; similarly, activation can be measured using antibodies specific for active conformations of cell surface molecules (Schmitz et al. 1998). Flow cytometry can also be used for the detection of platelet-reacting antibodies in patients with immune thrombocytopenic purpura or drug-induced thrombocytopenia, which is sensitive but nonspecific (Romero-Guzman et al. 2000). Mepacrine, which is taken up in dense granules, can be used to measure number of dense granules, platelet signaling, and granule release function (Wall et al. 1995). P-selectin, which is newly expressed on the platelet surface after activation, can be used to measure platelet alpha granule release (Fig. 1.6). Flow cytometry has the benefit of requiring a relatively small amount of blood compared to aggregation studies. However, platelet flow cytometry, like aggregometry, is generally performed only in specialized medical centers as it requires specialized instruments and skilled technologists. This can make performing these studies logistically very difficult because platelet function studies by flow cytometry (such as alpha or dense granule release studies) should be performed within 1 hour of venipuncture because of progressive activation of platelets during in vitro storage. However, interrogation of surface glycoproteins, such as is used for making a diagnosis of Glanzmann thrombasthenia, can be measured in specimens up to 24 hours post-collection (Michelson 1996; Michelson 2006; Kottke-Marchant 2008).

Thromboelastography

Thromboelastography and thromboelastometry are similar techniques used to monitor the viscosity and elasticity of blood clots and can simultaneously measure coagulation, platelet function, and fibrinolysis through analysis of the viscoelastic properties of clotting blood. There are two commercially available platforms available for clinical use: thromboelastography (TEG; Haemoscope/Haemonetics Corp, Niles, IL, USA) and rotational thromboelastometry (ROTEM; TEM International, Munich, Germany) (Bolliger

Table 1.3 Summary of platelet disorders with characteristic laboratory findings

	Defect	Platelet count	Morphology	Characteristic aggregometric findings	Other platelet assay results	Comments
Surface glycoprotein disorders						
Glanzmann thrombasthenia	Deficient or defective GPIIb/IIIa receptor (AR)	Normal	Normal	Absent with all agonists except ristocetin	Prolonged CT Decreased GPIIb/IIIa receptor by flow cytometry	Similar picture with afibrinogenemia
Bernard–Soulier syndrome	GPIb/V/IX receptor (incompletely recessive AR)	Decreased (40,000–1,000,000/μL)	Giant platelets high MPV	Normal with all except ristocetin	Prolonged CT Decreased expression of GPIb/V/IX receptor by flow cytometry	Mucocutaneous bleeding out of proportion to thrombocytopenia. Surgical bleeding risk
Velocardiofacial syndrome	Deletion of 22q11.2 (GPIb beta gene)	May be decreased	Giant platelets high MPV	Absent with all agonists except high dose ristocetin		Bleeding uncommon
Collagen receptor deficiency	Defect in collagen receptors (GPIa/IIa, GPVI)	Normal	Normal	Absent with collagen and low dose ristocetin		Very rare
Platelet-type/pseudo-von Willebrand disease	Gain-of-function mutation in GPIb receptor → decreased HMW vWF multimers	Decreased	Normal (rarely giant platelets)	Increased with low dose ristocetin. Normal with all others	Prolonged CT	Similar picture in Type 2B vWD; differentiate by *VWF* gene sequencing, mixing studies, and platelet aggregation by adding cryoprecipitate to PRP
Storage-pool disorders						
Gray platelet syndrome	Deficiency of alpha granules	Often decreased	Large, gray-colored (agranular) platelets	May be decreased with thrombin and collagen	Decreased alpha granules in EM	Marrow and pulmonary fibrosis, splenomegaly Mild lifelong bleeding
Wiskott–Aldrich syndrome and X-linked thrombocytopenia	Defect in Wiskott–Aldrich syndrome protein gene (XR)	Decreased	Uniformly small, low MPV	Absent with ADP, epinephrine and collagen		Immune deficiency, recurrent infection, often decreased dense granules, increased bleeding risk

(continued)

Table 1.3 (continued)

	Defect	Platelet count	Morphology	Characteristic aggregometric findings	Other platelet assay results	Comments
Dense granule deficiency	Defective dense granules. Can be isolated or as part of hereditary syndromes	Normal	Usually normal	May have absent second wave with ADP, epinephrine, and decreased response to AA and collagen. Normal in 25 % of patients	Decreased mepacrine uptake by flow cytometry	Hermansky–Pudlak, Chediak–Higashi, thrombocytopenia with absent radii (TAR) syndromes
Activation disorders						
Signaling defects	Defects of cyclooxegenase, phospholipase C pathways	Normal	Normal	Decreased primary aggregation with epinephrine and collagen, absent secondary with ADP	Normal mepacrine uptake	Decreased granule release without granule deficiency
Secretion defects	No release in dense granules	Normal	Usually normal	Decreased primary aggregation and absent secondary aggregation		
Scott syndrome	Defective "flip" of membrane phospholipids to outer surface during activation	Normal	Normal	Normal	Abnormal platelet procoagulant activity	Decreased binding of activated coagulation factors, decreased microparticle formation
Drug-induced disorders						
ASA/NSAIDs	Cyclooxegenase inhibition: ASA-reversible; NSAIDs-irreversible	Normal	Normal	Absent second wave with ADP; decreased or absent with others except ristocetin	Prolonged CT (often collagen/ epinephrine cartridge)	Similar findings with COX-1 deficiency or functional abnormality → defective TxA_2
Thienopyridines (e.g., clopidogrel)	Inhibition of ADP receptor ($P2Y_{12}$)	Normal	Normal	Absent second wave with ADP	Prolonged/ normal CT	Similar picture in very rare cases of congenital ADP receptor impairment
GPIIb/IIIa inhibitors (e.g., abciximab, eptifibatide, tirofiban)	Inhibition of GPIIb/IIIa receptor			Decreased or absent for all except ristocetin	Prolonged CT	

Others						
Myeloproliferative neoplasms	Clonal neoplasm	Increased, often >1,000,000/μL; sometimes decreased	Variable size and morphology; occasional hypogranularity	Variable. Decreased with epinephrine in ET	May show reduced GPIIb/IIIa expression by flow	Bleeding or thrombosis
MYH9 disorders	Mutations in non-muscle myosin heavy chain IIA protein	Decreased (60,000–100,000/μL)	Giant platelets, high MPV, neutrophilic inclusions	Normal		Includes May–Hegglin anomaly, Sebastian, Fechtner, and Epstein syndromes. Mild bleeding

CT closure time, *HMW* high molecular weight, *vWF* von Willebrand factor, *GP* glycoprotein, *AR* autosomal recessive, *XR* X-linked recessive, *ET* essential thrombocythemia, *MPV* mean platelet volume

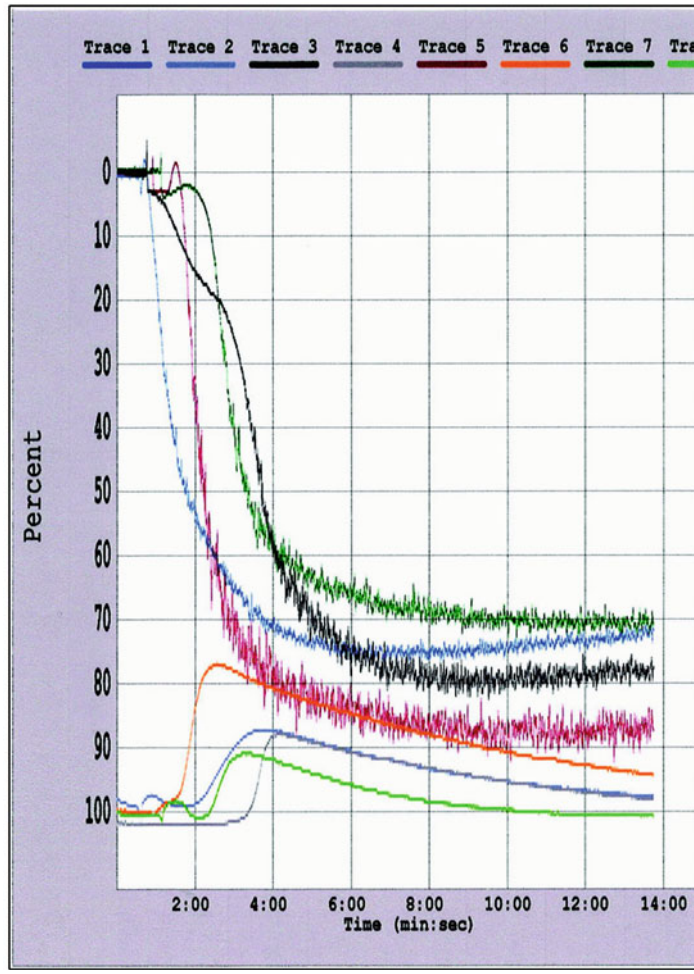

Fig. 1.5 Example of normal lumiaggregation with simultaneous measurement of platelet aggregation and stimulated ATP release by dense granules during platelet aggregation. Platelet aggregation, which is shown at the *top*, is measured by maximal percentage of aggregation, and agonists in this diagram include ADP (5 μM; *blue*), collagen (2 μg/mL; *red*), arachidonic acid (500 μg/mL; *green*), and epinephrine (10 μM; *black*). The corresponding ATP aggregation to ADP (*light blue*), collagen (*orange*), arachidonic acid (*light green*), and epinephrine (*gray*) is shown at the *bottom*

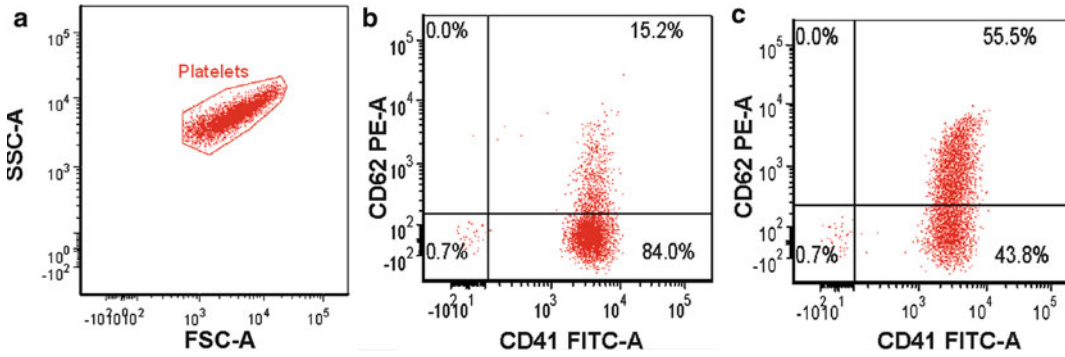

Fig. 1.6 Example of normal platelet flow cytometry. (**a**) Platelet population is gated by the platelet size (forward scatter: FSC) and granularity (side scatter: SSC) in whole blood. (**b**) Basal P-selectin (CD62): resting platelets express surface glycoproteins GPIIb/IIIa (CD41), but do not express high levels of P-selectin (CD62). (**c**) Stimulated P-selectin (CD62): upon stimulation with ADP, most platelets release granules from alpha granules and express high levels of P-selectin (CD62) on the platelet membrane surface

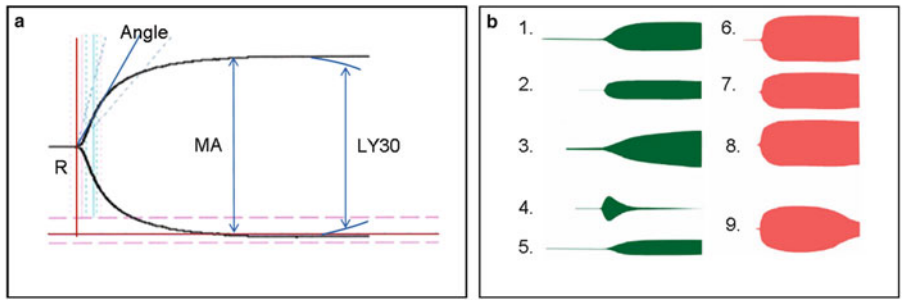

Fig. 1.7 Thromboelastography (TEG). (**a**) Diagram of TEG tracing. R, reaction time until initiation of clotting; angle, degree of the strength of clot growth; MA, maximum amplitude indicative of maximum strength or stiffness of developed clot; LY30, percent lysis 30 min after maximum amplitude. (**b**) Examples of abnormal tracing pattern: *1*. low clotting factors, *2*. low platelet function, *3*. low fibrinogen level, *4*. primary fibrinolysis, *5*. hypocoagulable state, *6*. platelet hypercoagulability, *7*. enzymatic hypercoagulability, *8*. platelet and enzymatic hypercoagulability, and *9*. secondary fibrinolysis (Image of the TEG® Thromboelastograph® Hemostasis tracings is used by permission of Haemonetics Corporation. TEG® and Thromboelastograph® are registered trademarks of Haemonetics Corporation in the USA, other countries, or both)

et al. 2012). The test involves measuring tensile force generated between a plastic cup of recalcified citrated whole blood and a metal pin immersed within it which increases as blood clots. In TEG, the cup rotates around the pin, while the pin rotates in a stationary cup in ROTEM. Clot activator is added to the specimen, and as clot forms the torque of the rotating component is transmitted to the stationary component and is plotted as a kinetic curve. The instrument measures time until initiation of clotting (*R*), time until a fixed level of clot firmness (*K*), degrees of the strength of clot growth (angle), maximum amplitude (MA) indicative of maximum strength or stiffness of developed clot, and percent lysis 30 min after MA (LY30) (Bolliger et al. 2012). Figure 1.7 shows a diagram of thromboelastography tracing and examples of abnormal tracing pattern such as factor deficiency, anticoagulant therapy, fibrinolysis, hypercoagulability, and consumptive coagulopathy status. The basic test utilizes a contact activator to initiate clotting (kaolin in TEG, ellagic acid/phospholipids for ROTEM), but others, such as tissue factor, are available. The test can be modified through the use of various activators and inhibitors to interrogate different components of both hemostasis and clot lysis (Chen and Teruya 2009). Although the basic test is insensitive to aspirin or thienopyridines, modifications have been developed to monitor such antiplatelet therapy or heparin reversal (Swallow et al. 2006). This near-point-of-care test can be used intra- and perioperatively (results in 20–30 min) to guide transfusion therapy, and transfusion protocols which incorporate TEG have been shown to decrease bleeding in patients with massive transfusion (Afshari et al. 2011). TEG is sensitive to hematocrit and platelet count, and thus far methods are not well standardized, but efforts are ongoing to improve this area (MacDonald and Luddington 2010; Kitchen et al. 2010).

Point-of-Care Tests

Currently there are a number of commercially available point-of-care (POC) tests for platelet function which provide rapid results, use small sample volumes, and require little to no specimen preparation (Table 1.4). POC tests also have the advantage of bypassing some of the logistical issues in platelet function testing such as timing and specimen transport. However, these tests are problematic in a variety of ways including a lack of standardization of methods, and performance by untrained personnel, in addition to having expensive reagents, quality control materials, and consumables, resulting in a relatively high cost-per-test (Gardiner et al. 2009). POC tests are generally used to predict intraoperative bleeding or monitor antiplatelet therapy. Some are designed

Table 1.4 Summary of commercially available point-of-care (POC) tests for platelet function

	Analysis method	Use	Specimen volume, anticoagulant	Advantages	Disadvantages
Plateletworks® (Helena Laboratories)	Comparison of free platelet count in blood with and without agonist	Monitor antiplatelet therapy Predict bleeding	1 mL, EDTA or special tubes	Results in 5 min No sample preparation	Test must be done within 10 min
Verify Now® (formerly Ultegra, Accumetrics)	Turbidimetric (light transmittance) aggregometry	Monitor antiplatelet therapy	2 mL, citrate or heparin, depending on test	Results in 5–10 min No sample preparation	Test must be done within 4 h
Multiplate® (Roche)	Impedance aggregometry	Monitor antiplatelet therapy Identify patients at risk for bleeding	0.3 mL, hirudin	Results in 10 min	Not currently available in US
Sonoclot® (Sienco)	Impedance of ultrasonic vibration as clot forms	Cardiac and transplantation surgery	400 µL, whole blood	Global view of coagulation, platelet function, and fibrinolysis	May take up to 45–60 min
Impact™ Cone and Plate (Matis)	Image analysis of adhesion and aggregation under shear stress	Diagnosis of platelet defects Monitor antiplatelet therapy	130 µL, citrate	Results in 6 min High shear stress	Clinical point-of-care assay not currently available in USA

to assess the effect of specific antiplatelet therapies using reagent cartridges based on the drug class of interest. Others such as the Sonoclot and Impact Cone and Plate(let) Analyzer provide a more global view of coagulation, platelet function, and fibrinolysis, similar to TEG (Pakala and Waksman 2011; Zeidan et al. 2007).

Platelet Disorders

Specific platelet disorders will be discussed in detail in other chapters of this book. The following table summarizes characteristic laboratory findings in different platelet disorders (Table 1.3).

Role of the Pathologist in the Hemostasis Laboratory and Clinical Hemostasis Consultation

Role of the Pathologist in the Hemostasis Laboratory

In the United States, laboratories are regulated under the Clinical Laboratory Improvement Amendments (CLIA), passed by Congress in 1988. This act established quality standards for laboratory testing to ensure the accuracy, reliability, and timeliness of patient test results (CLIA Regulations). Laboratories are accredited according to CLIA standards by deemed organizations, such as the College of American Pathologists (CAP).

The CLIA act established the role of the Laboratory Director, which is often performed by a pathologist. According to CLIA regulations, the Director of laboratories, such as the hemostasis laboratory, is responsible for the overall operation of the laboratory and ensures compliance with applicable regulations (CLIA Regulations). Laboratory Directors ensure that the testing systems employed provide quality laboratory service in the preanalytic, analytic, and postanalytic phases of testing. Laboratory Directors review and sign off on all new and substantially changed policies and procedures prior to implementation and whenever there is a change in Laboratory Director. The Laboratory Director establishes a quality management process in the laboratory and monitors key performance indicators, such as quality control and quality assurance, with corrective action, as necessary. For accreditation, laboratories must also perform proficiency testing, where laboratories test unknown specimens, with their results compared against peer groups. Laboratory Directors must make provisions for proficiency testing and review the laboratory's results. In addition, the Laboratory Director must ensure that there are appropriate numbers of trained individuals to perform testing and that the physical facility is adequate.

Role of the Pathologist in the Clinical Hemostasis Consultation

In hemostasis testing, there are many unique issues which may make a pathologist's consultation helpful. Apart from the rapidly expanding knowledge of both bleeding and thrombotic disorders and a wide test menu, hemostasis testing is very sensitive to preanalytic issues (hemolysis, fill volume, time, temperature) and the interference of many commonly prescribed drugs.

The pathologist can serve an important role in the evaluation of a patient for a bleeding or thrombotic disorder. Several hemostasis laboratories in major medical centers have established consultative hemostasis testing services where clinicians are offered a panel or battery of initial hemostasis tests (Marques et al. 2011; Hayward et al. 2012). At the Cleveland Clinic, our consultative hemostasis testing service offers several interpretive panels, such as an elevated aPTT panel, an elevated PT/aPTT panel, a lupus anticoagulant panel, a hypercoagulation panel, and a von Willebrand disease panel.

When such a panel is ordered on a patient, the specimen is collected and processed and multiple aliquots are prepared and stored. A specialized physician, such as a hematopathologist, evaluates the initial results and then adds additional tests from the stored specimen, based on the patient's clinical scenario, medication history, and a laboratory-defined algorithm. When all testing is complete, the pathologist interprets the testing

results and issues a personalized written diagnostic report, indicating the cause and significance of the hemostasis abnormality.

This consultative hemostasis testing service may improve the efficiency and quality of patient care as it is designed to streamline the testing type and cost, where only the needed tests are performed. It can improve patient experience by decreasing the need for multiple phlebotomies and potentially decreasing the sample volume needed for diagnosis. It may also improve the patient outcome by preventing misdiagnosis due to the effect of interfering drugs; for example, where the effect of a direct thrombin inhibitor can be detected before erroneously diagnosing a lupus anticoagulant. In one such consultative hemostasis testing service, 77 % of surveyed ordering physicians felt that the service saved time to diagnosis, 78 % felt that it impacted the differential diagnosis, 72 % felt that it prevented misdiagnosis, while 72 % felt that it reduced the number of tests performed (Laposata et al. 2004).

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Easy Bruisability

2

Alan Lichtin and Anthony P. Fernandez

Easy Bruisability

This chapter will focus on patients who are referred to a hematologist because they complain about easy bruising. This is a very common reason for a consult. Sometimes, based on history alone, one can determine the cause of the problem. Other times, even after extensive testing, the answer remains elusive. There are several excellent reviews of this topic (Kessler et al. 2007; Zumberg and Kitchens 2007).

History

Many patients with lifelong bruising are so used to the problem that they will not complain, but when they enter into a relationship, the new boyfriend/girlfriend/spouse/doctor will become concerned and send the patient to you. A person who never had easy bruisability before and now starts having the problem, will usually develop such a level of concern that they will refer themselves to you, or go to a family practitioner/internist/gynecologist, and that doctor may or may not perform tests that probably will not answer the question as to why the easy bruisability is occurring, and thus, they see you.

"Easy bruising" covers many types of manifestations of bleeding in the skin. "Black and blue marks" or ecchymoses are seen in a wide array of platelet, coagulation factor, and collagen disorders. Petechiae, often over the legs, usually are from thrombocytopenia. But other potential causes of petechiae on the legs include a benign condition called Schamberg's capillaritis and chronic venous insufficiency. Purpura, may be flat or flush with the skin surface or raised, the so-called palpable purpura seen with vasculitis (in general), leukocytoclastic vasculitis (see Fig. 2.7), or cryoglobulinemia (see Fig. 2.11) (Dupin et al. 1995). Henoch Schoelein purpura is a type of purpura which is usually palpable, as well (see Fig. 2.8). Painful bruises may be seen in coagulation factor deficiencies, often associated with deeper bleeding or soft tissue bleeding. When pain precedes the onset of a noticeable bruise, one may be dealing with auto-erythrocyte sensitization, a syndrome of cryptic etiology. Location of bleeding, such as the periorbital purpura seen in amyloidosis (Eder and Bitterman 2007), is so distinctive that the astute clinician can take one look at the person and know the underlying disease (see Fig. 2.13). Disorders of blood vessels, usually inflammatory, can cause skin changes that appear as bruises.

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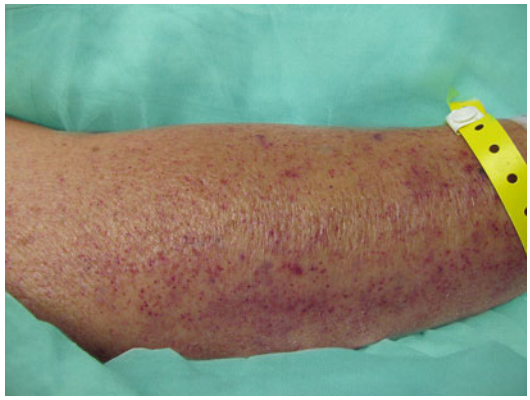

Fig. 2.1 Petechiae. This patient had acute onset of idiopathic thrombocytopenic purpura, with a platelet count of 2,000/μL

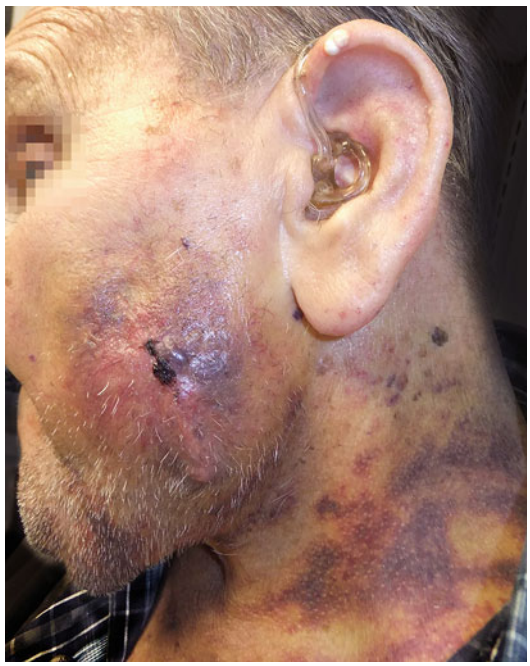

Fig. 2.2 Post operative hematoma. Bruising and ecchymosis from adjacent site of surgery

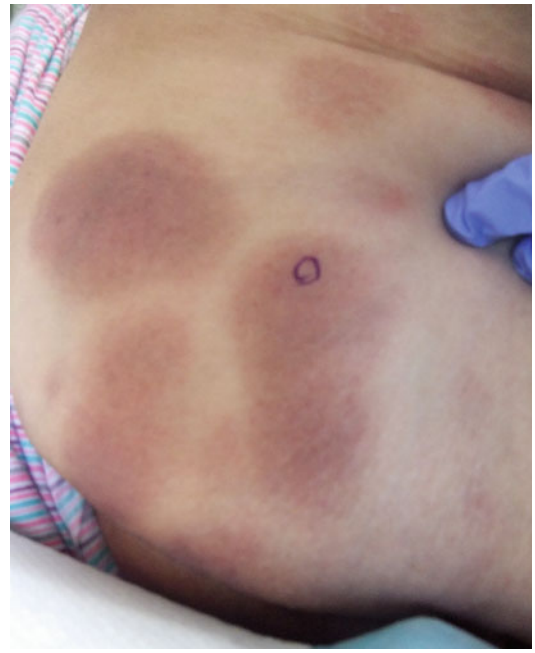

Fig. 2.3 Fixed drug eruption. This patient had 2 weeks of exposure to fluconazole and developed these lesions

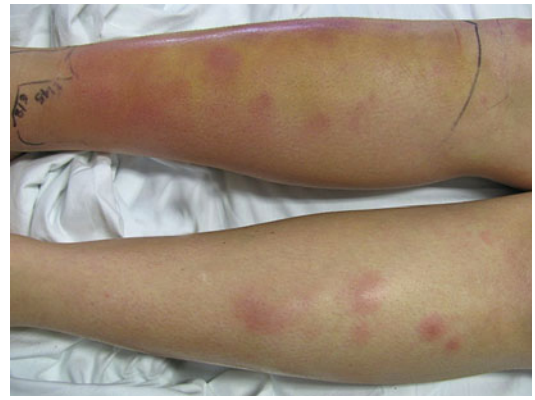

Fig. 2.4 Erythema nodosum. This 22-year-old woman had a viral illness and developed these painful lesions. There was no oral contraceptive use, nor underlying disease

Medication history is very important. If the bruising did not appear until a new medication was started, like aspirin, clopidogrel, dipyridamole, coumadin (warfarin), or other anticoagulant, one can find fault easily. However, some people will not consider their over-the-counter use of nonsteroidal anti-inflammatory agents as a "medication," and the careful clinician has to ask specifically about all prescribed drugs, as well as over-the-counter or those purchased online, including nutritional supplements.

Family history may give keen insight into the causes of easy bruising. Whenever a patient says something like, "I have a sister/parent/cousin with the exact same bruising," careful clinicians will try to obtain a pattern. Is the problem sex-linked? Does it skip a generation? What occurred

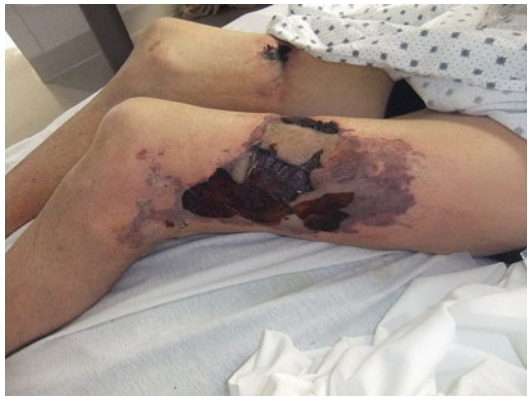

Fig. 2.5 Calciphylaxis. This is vascular calcification, thrombosis, and skin necrosis, seen primarily in dialysis patients and in patients with recent renal transplants. There is no specific treatment, but it can be prevented with control of phosphate and calcium levels. It is usually a fatal condition

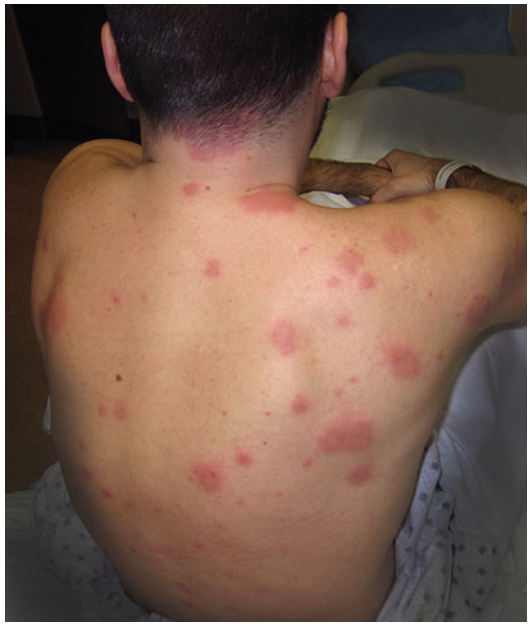

Fig. 2.6 Sweet's syndrome (*acute febrile neutrophilic dermatosis*) is characterized by acute onset of erythematous plaques and is occasionally seen in association with underlying myelodysplasia and acute leukemia. This patient, however, was a 29 year old man who became acutely jaundiced, and work-up revealed IgG4 related cholangitis. He developed this rash as part of his presentation

in affected individuals during periods of greater hemostatic stress, such as surgery, especially if the patient in front of you has not yet had that stress in their lives?

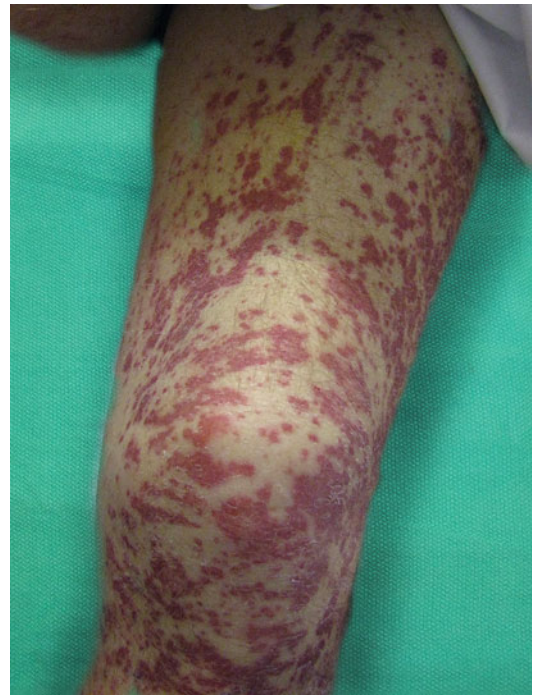

Fig. 2.7 Leukocytoclastic vasculitis. This occurred in the setting of chronic hepatitis C infection with thrombocytopenia

Clinical Vignette

A 75-year-old woman comes to your office for easy bruising. Over the past few months, she noticed bruises on her skin, and she did not remember any trauma that led to them. They occurred on her arms and legs primarily, and did not appear on her face or trunk.

She was in excellent health and still worked as a bookkeeper. She had three children. There was no family history of any type of bleeding disorder.

She took a thiazide diuretic for mild hypertension for ten years, and was on no other medications, especially aspirin, which she said caused her to have hives when she once took it in her twenties, so she avoided it.

She had had an appendectomy as a teenager and had no bleeding after it.

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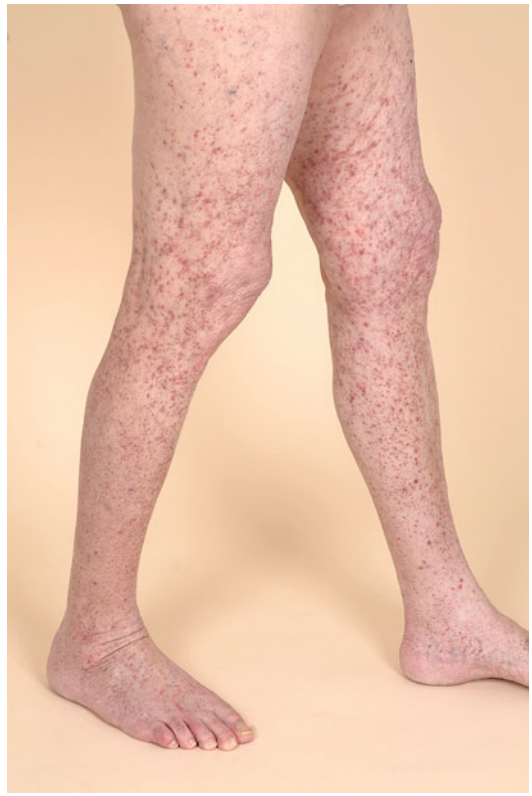

Fig. 2.8 Henoch-Schonlein purpura (HSP). This 69-year-old woman had rash, fatigue, fevers for 2 weeks. Biopsy showed leukocytoclastic vasculitis. Direct immunofluorescence showed perivascular IgA, consistent with HSP. Urinalysis showed 2^{+} blood and 30 mg/dL protein

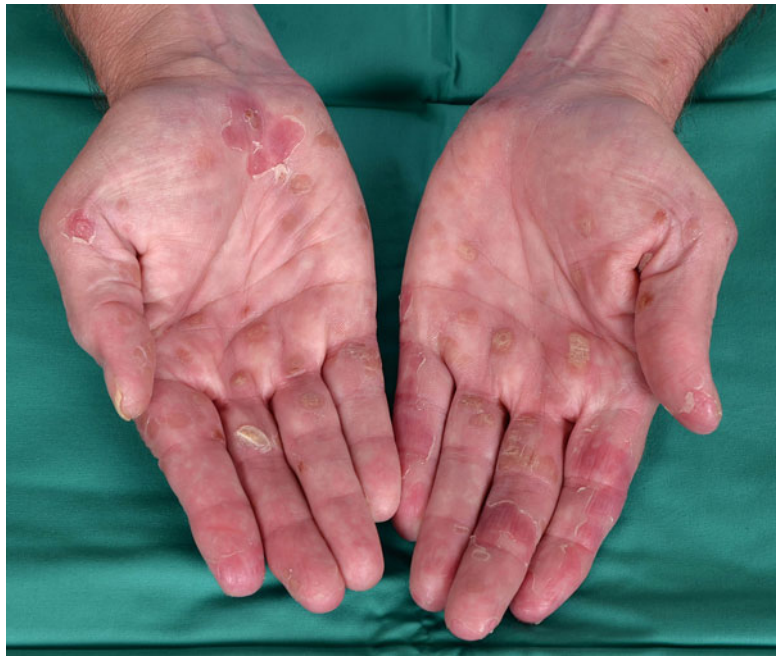

Fig. 2.9 Erythema multiforme. This 70-year-old man had biopsy proven recurrent erythema multiforme, not related to herpes simplex, or any other known virus. This condition is thought to be due to immune complex deposition (IgM primarily) in the microvasculature of the skin, after viral infections or drug exposures

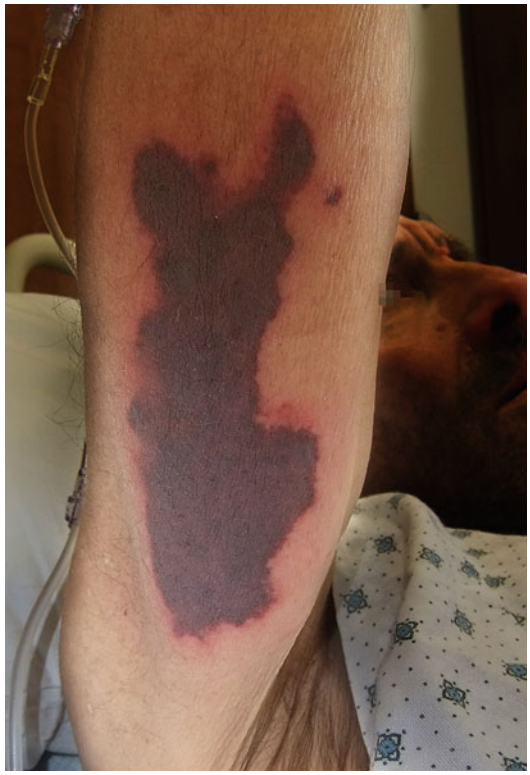

Fig. 2.10 Levamisole-induced vasculitis. This young man had recently snorted cocaine that was cut with levamisole

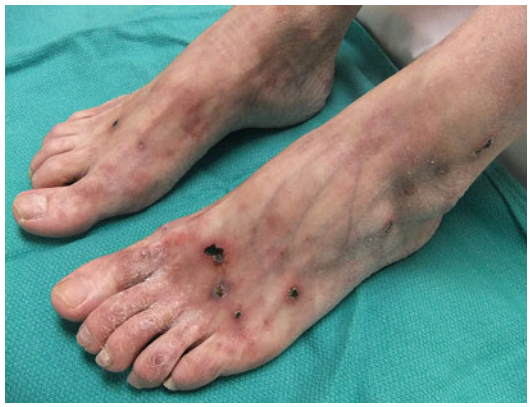

Fig. 2.11 Cryoglobulinemia. A 54-year-old woman with monoclonal gammopathy of undetermined significance (MGUS) and cryoglobulinemic vasculopathy. There was no underlying autoimmune disease or hepatitis C infection. Rheumatoid factor was normal. This was most consistent with Type 1 cryoglobulinemia

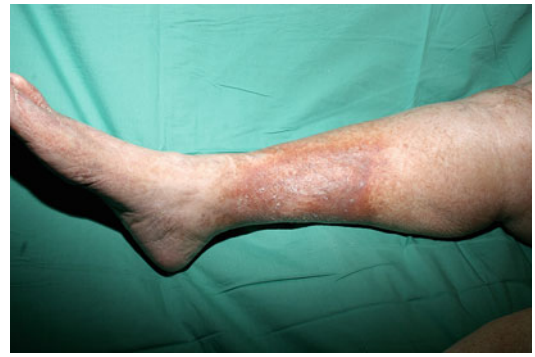

Fig. 2.12 Lipodermatosclerosis. This painful inflammation of subcutaneous fat (panniculitis) is often confused with cellulitis

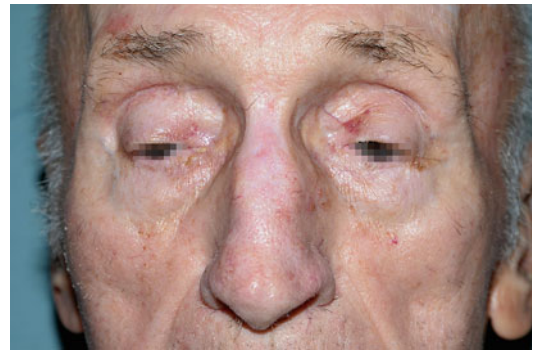

Fig. 2.13 Amyloidosis. Periorbital purpura is a common presenting sign in this condition

On exam, there were four bruises scattered over her arms and legs. They had a typical appearance of an ecchymosis, with fading coloration in older ones. There were no breaks in the skin, and there was no excoriation.

The rest of the examination was normal.

Her laboratory data demonstrated a normal platelet count, as well as a normal complete blood count and differential, normal PT, PTT, and fibrinogen, and her platelet function screen (PFA-100) showed values just above the normal range. Platelet aggregation studies demonstrated slightly below normal values for ADP-induced aggregation, and epinephrine-induced aggregation.

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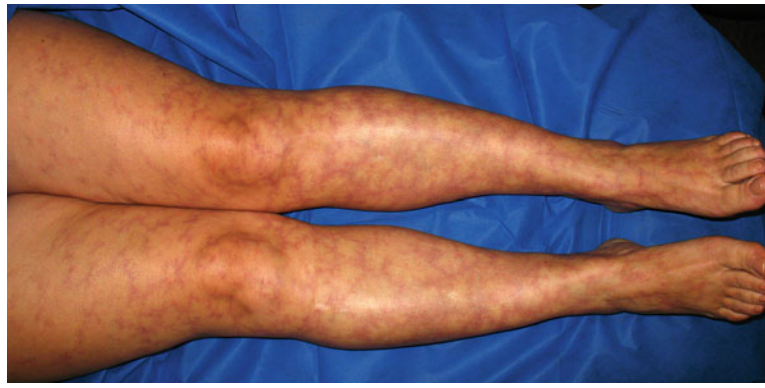

Fig. 2.14 Livedo reticularis. This person had mottling of the skin and biopsy proven leukocytoclastic vasculitis. Livedo reticularis can be idiopathic and completely benign, common in women, and it can get worse in cold temperatures. It is caused by swelling in the venules, secondary to obstruction of the capillaries by thrombi. It can also be seen in many other disorders, especially rheumatologic conditions, anti-phopholipid antibody positivity, and cancer

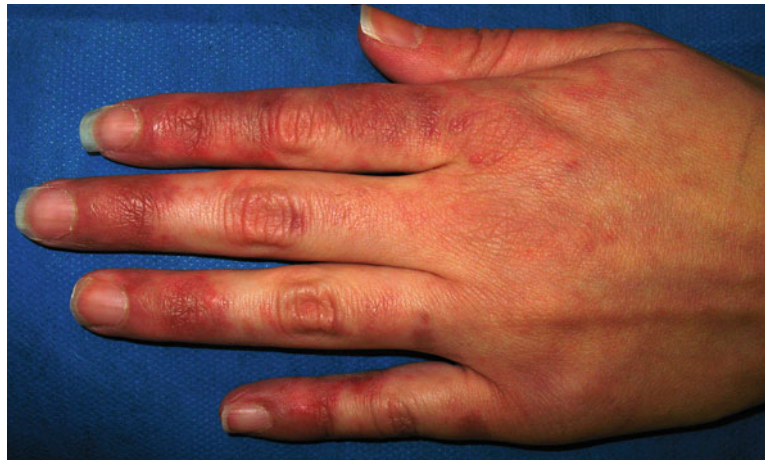

Fig. 2.15 Chilblain lupus erythematosus. Painful purpuric fingertips in a young woman. Serologic workup revealed positive anti-SSA antibodies

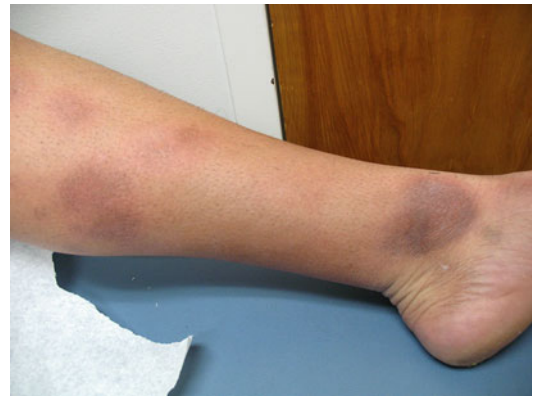

Fig. 2.16 Lichen aureus. Pigmented purpuric dermatosis in a 25-year-old man. Asymptomatic bruise-like patches. Biopsy revealed capillaritis with extravasated red blood cells, consistent with pigmented purpuric dermatosis. Morphology of patches in this case is most consistent with a diagnosis of lichen aureus

It was thought that, perhaps, the thiazide diuretic might be the cause of these abnormalities, but that would not explain the recent onset of the bruising, since she had been on the anti-hypertensive for years, and the bruising was just recent.

The patient is brought back to clinic for a more careful history, asking particularly if she is taking any other medication, or over the counter medicines. She confesses that she has been taking her husband's fish oil preparation lately, because she read an article about its health benefits. She did not mention it originally, because she didn't really feel it was a medication in the initial visit.

It was advised for her to stop the fish oil, and within one month, the bruising disappeared. She was tested again for platelet function screen and platelet aggregation, and now, all values were in the normal range.

Examination

In the previous few figures, one can see various types of easy bruising and their causes. Some of these were mentioned earlier in this chapter.

Laboratory Evaluation

The screening coagulation tests that should be performed on all patients who come for an evaluation of easy bruising include PT, PTT, fibrinogen, complete blood count (cbc) and differential, platelet count, a platelet function screen (either PFA-100 or platelet agregation studies), and a comprehensive metabolic panel (CMP). Any abnormality on these screening tests should be pursued to determine if they demonstrate the cause of the easy bruising.

A prolonged PT might indicate Vitamin K deficiency, or FVII deficiency or an inhibitor to FVII; elevated PTT may indicate FXII, FXI, FIX, or FVIII deficiency or inhibitors to these, or Von Willebrand's disease. Prolongation of both PT and PTT may indicate deficiencies of, or inhibitors to FX, FV, FII, and fibrinogen, or Vitamin K deficiency, or liver disease. A low platelet count can be a cause for easy bruising and the reasons for a low platelet count are myriad. Essentially, they can be divided into underproduction of platelets in the marrow, overdestruction of platelets in the circulation or sequestration of platelets in the spleen. Bruising can occur in individuals with elevated platelets in myeloproliferative disorders, especially essential thrombocytosis, because the platelets are dysfunctional.

A normal platelet count accompanied by an abnormal platelet function screen (PFA-100), or platelet aggregation studies, would indicate a platelet function defect. Some of these are congenital, like Glanzmann's thrombasthenia (Glanzmann 1918; Sebastiano et al. 2010) and alpha (Levy-Toledano et al. 1981) and dense granule deficiency, and others are acquired, especially after exposure to aspirin and clopidogrel, or in renal failure. A low fibrinogen level may be a cause for easy bruising—this, too, can be congenital, or acquired, as in DIC, or an acquired abnormality, such as dysfibrinogemia associated with cancer (Cunningham et al. 2002). Abnormal liver function tests on CMP may indicate liver disease, which can often be associated with decreased levels of clotting factors that are manufactured there.

The most difficult cases of easy bruising are those for which all screening tests are normal and the bruising continues to be apparent.

Disorders of Factor XIII (Hsieh and Nugent 2008) and fibrinolysis (Ferro et al. 2009) can cause easy bruising, though these are usually lifelong. Disorders of connective tissue, such as Ehlers Danlos Syndrome (DePaepe and Malfait 2004) can present with easy bruising. On history, these individuals can describe their hyperlax joints and odd tricks they could do with their joints.

Bruising with Normal Screening Tests

Factor XIII Deficiency

Normal levels of fibrinogen, PT, and PTT can still be associated with bruising. This can be because Factor XIII, or fibrin stabilizing factor is not measured with the commonly used screening tests.

Patients with congenital Factor XIII deficiency can have severe hemorrhaging and poor wound healing. A typical historical piece of information is bleeding from the umbilicus soon after birth.

Factor XIII is a transglutaminase which stabilizes fibrin. It is a tetramer, with 2 A subunits and 2 B subunits. Factor XIII is activated by fibrin into Factor XIIIa. At the N-terminus of the A subunit, between the 37th and 38th amino acid, there is a thrombin specific cleavage point. A conformational change occurs, exposing a catalytic site that acts on fibrin to form gamma-glutamyl—epsilon-lysylamide cross-links between fibrin molecules to form an insoluble clot (Komaromi et al. 2011). The way the laboratory measures Factor XIII level is by a clot solubility test (Bhattacharya et al. 2005). Thrombin-treated plasma clots are placed in 5 Mol urea or 1 % mono-chloracetic acid. If the clot dissolves rapidly, it proves the fibrin is not cross-linked properly and Factor XIII deficiency is demonstrated. The clots from people with normal levels of Factor XIII remain insoluble for >24 h. Factor XIII inhibitors can be diagnosed by mixing normal plasma with patient's plasma and then performing the clot solubility test. There are quantitative tests for determining how much Factor XIII activity is present, using a Factor XIII quantification assay (Hsieh and Nugent 2008).

Humans only need 5 % Factor XIII activity to maintain normal cross-linking of fibrin. The half-life of Factor XIII is particularly long at 10 days. Factor XIII is found in plasma and in platelets. Indeed, half of the entire body's Factor XIII is stored in platelets. Infants born with Factor XIII deficiency typically bleed from the umbilical stump, as mentioned above. Spontaneous mucous membrane, soft tissue, and intracranial bleeding can occur in individuals with severe deficiency (<1 % activity). Women with FXIII deficiency can have spontaneous abortions, and men with it can have low sperm counts. Once diagnosed and treated with FFP, some patients form alloantibodies to the infused Factor XIII molecules.

Idiopathic Factor XIII antibodies are probably the most common cause of inhibitors to FXIII (Harris et al. 1985). Certain drugs (isoniazid, phenytoin, and penicillin) can induce Factor XIII antibodies (Otis et al. 1974). Lowered levels of Factor XIII have been associated with certain diseases, such as inflammatory bowel disease and Henoch-Schonlein purpura (Kamitsuji et al. 1987).

Alpha$_2$-Plasma Inhibitor Deficiency

Plasmin accelerates the process of fibrinolysis. An inhibitor to plasmin keeps this in check. If one is deficient in this inhibitor, then ongoing, sustained, and increased levels of fibrinolysis are maintained (Koie et al. 1978). These individuals can present with unexplained easy bruising, menorrhagia, epistaxis, hematuria, and even hemarthroses. PT, PTT, platelet function tests, and thrombin times are normal, though, sometimes, the fibrinogen level can be low.

Alpha$_2$-plasmin inhibitor deficiency is inherited as an autosomal recessive defect. The gene for alpha$_2$-plasmin inhibitor is located on chromosome 17 (Carpenter and Mathew 2008). It is also called a serine protease inhibitor (serpin). Since it is manufactured in the liver, individuals with liver failure may have an acquired deficiency of it. This causes decreased inactivation of plasmin and an increase in fibrinolysis. It is difficult to distinguish between the accelerated fibrinolysis of disseminated intravascular coagulation (DIC) that can be associated with cirrhosis or liver failure, versus alpha$_2$ plasmin inhibitor deficiency. Alpha$_2$ plasmin inhibitor deficiency has also been associated with solid tumors, acute promyelocytic leukemia, other causes (besides liver failure) of DIC, and amyloidosis.

Diagnosis can be hinted at by noting an accelerated euglobulin lysis time. Specific assays for alpha$_2$ plasmin inhibitor are available and should be ordered when a deficiency state is suspected. Once diagnosed, treatment usually includes anti-fibrinolytic agents such as epsilon-amino caproic acid.

Factitious Bruising

Many individuals with mental illness try to draw attention to themselves by self-induced trauma (Koie et al. 1978; Komaromi et al. 2011; Levy-Toledano et al. 1981). Others may take warfarin or other blood thinners surreptitiously, but when they present with bruising/bleeding, one can see abnormal coagulation testing.

An entity called erythrocyte auto-sensitization syndrome used to be part of coagulation textbooks years ago, but has fallen out of favor as a true entity (Ratnoff 1989). These individuals often have a history of multiple previous surgeries. It was thought that multiple exposures to blood or red cell stroma in subcutaneous tissues led to an altered immune response. The clinical manifestations were usually that of pain in the skin where no bruising was first seen, followed by an ecchymosis or hematoma (Uthman et al. 2000). Psychiatric disease would often accompany this clinical presentation. These individuals often had a major psychic trauma in their own lives and by history, the bruising started after this traumatic event. An evocative test is to sit the patient down, and very suggestively state that a test for the definitive diagnosis of their bruising problem will be performed. Take 0.1 cc of sterile saline in one syringe and 0.1 cc of the patient's own EDTA anticoagulated blood in another and inject them subdermally (like a TB skin test) into the skin or the volar surface of each arm. The person performing the test should tell the patient that it is expected that the arm that has the blood injected under the skin should develop an impressive ecchymosis and the saline should not. A positive test would indeed show a difference between the larger ecchymosis formation in the injection site of the autologous blood versus the saline one. If positive, reassurance is given that there is no severe coagulopathy and psychiatric counseling is recommended (personal communication, George Hoffman, M.D.).

Amyloidosis

Deposition of amyloid in dermal vessels can lead to purpura. The vascular integrity is compromised by the amyloid and the gentlest of trauma can cause bruising. For reasons attributable to the delicacy of the skin location, periobital purpura is almost pathognomonic of amyloid. This is true especially after Valsalva maneuvers, leading to the common clinical setting of "post-proctoscopic periorbital purpura," or the 4 P's (see Fig. 2.13) (Eder and Bitterman 2007).

The location of the amyloid deposition is usually between the endothelium and the basement membrane. Depending on what organ is most affected, one may see central nervous system bleeding, GI bleeding, or skin bleeding. In some individuals, the amyloid inhibits Factor X activity (Choufani et al. 2001), compounding the capillary fragility aspect of bleeding.

Connective Tissue Disorders

A not infrequent consult may arrive in one's office with a history of severe bleeding postoperatively, finally stopping after many units of red cells and an extensive work up has been undertaken with no apparent cause for the bleeding. Surgical hemostasis should come to mind when hearing this story (were all vessels properly ligated or coagulated?), but another possibility is that the tissues through which vessels flow have abnormal supporting capability. These individuals may be hyperlax, as in Ehlers Danlos Syndrome (EDS). They may have lifelong abilities to stretch their joints in unusual ways (touching their thumb to the volar surface of the distal forearm, putting their legs behind their neck while sitting on the floor). They may or may not complain of easy bruising, but a mild change in behavior, like taking aspirin or ibuprofen, or of circumstance, like surgical trauma, will tip the balance in favor of bleeding and they will complain of ecchymoses.

Ehlers Danlos Syndrome is actually several disorders, all related to defects in collagen synthesis (Remvig et al. 2009). The astute clinician will ask about any history of dislocated or subluxed joints. Exam will focus on hyperlaxity of joints, stretchy skin, fragile skin, tearing skin, and high or narrow palate. Mutations of col 1A1,

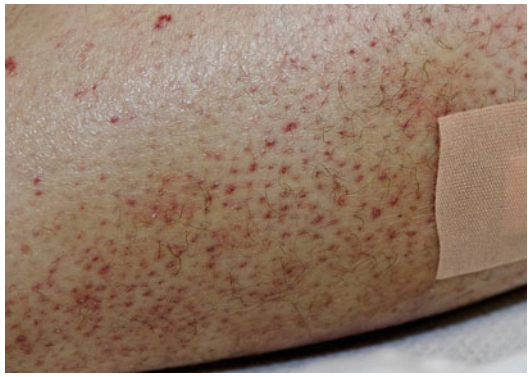

Fig. 2.17 Scurvy. Vitamin C deficiency, with perifollicular hemorrhages and the associated "corkscrew" hairs

col 1A2, and other genes can be assayed to prove that the syndrome exists in the patients. A skin biopsy and amino acid sequencing of the collagen may aid in typing the EDS (Uitto 2005).

The bruising from EDS is usually a nuisance and requires no treatment other than avoidance of trauma and avoiding medications like aspirin that could make bleeding worse. Children and young adults should be counseled not to show off their hyperlaxity because eventually, the joints can be damaged, leading to disabling arthritis. There is one variant of EDS, type IV, in which aneurysmal dilation of visceral vessels, like the splenic artery, can rupture and cause fatal bleeding (Harris et al. 1985; Morais et al. 2011; Yost et al. 1995).

Other collagen disorders that can cause easy bruising include Marfan's syndrome, osteogenesis imperfecta (OI), and pseudoxanthoma elasticum (PE). Marfan's is characterized by tall stature, dislocated lens, and aortic root aneurysm (DePaepe and Malfait 2004; Dietz 1993; Judge et al. 2004). OI is characterized by blue sclerae and frequent bone fractures (Rauch and Glorieux 2004; Barnes et al. 2010). PE is a disorder in which calcium deposits are found in lax skin and this causes fragmentation and mineralization of the elastic fibers. This disorder is caused by autosomal recessive mutation of the ABCC6 gene. This is found on the 16 p chromosome (Bercovitch and Terry 2004).

Scurvy (See Fig. 2.17)

Lack of Vitamin C intake can lead to scurvy (Smith et al. 2011). In this disorder, perifollicular hemorrhages are seen. Ecchymosis and bleeding spongy gums can occur. In the absence of Vitamin C, the individual cannot cross-link fibers of collagen at proline sites. This is because Vitamin C is necessary to convert proline to hydroxy-proline in order to facilitate the cross-linking structure of collagen. Once Vitamin C is given, there is remarkable and rapid improvement.

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Prolonged PT

3

Alan Lichtin

Introduction

It is unusual for "prolonged PT" to be the reason for a consult. More commonly, both PT and PTT are prolonged, or isolated PTT elevation is more common because there are more reasons for an elevated PTT compared with PT.

Remembering back to the coagulation cascade, the PT measures the extrinsic system. Factor VII is cleaved by tissue factor to act on factor X, in order to activate factor X. The primary reason why there would be an isolated PT prolongation usually involves a deficiency of, or rarely, an inhibitor to factor VII.

Clinical Vignette

You are asked to see a patient in the SICU, who is having some infectious problems after a routine cholecystectomy. She is a 54-year-old woman, who had normal coagulation tests (PT, PTT, and fibrinogen, as well as platelet count) prior to surgery. The cholecystectomy was done because of symptomatic stones, yet was done electively.

Post-op, amylase and lipase were normal, but she developed a wound infection and had positive blood cultures for Gram-negative rods and a brief episode of hypotension, for which she was on dopamine for less than 12 h. She had prolonged nausea and vomiting and was not eating or drinking, as well as being on IV antibiotics.

Six days into the admission, routine labs were drawn, and the prothrombin time was now 19 s, with INR of 1.9, PTT normal, complete blood count and differential showed WBC 12,100, with slight left shift, hemoglobin 10.9 g/dL, and platelets 456 k/μL. Her fibrinogen was 645 mg/dL.

The reason you are being asked to see her is the prolonged prothrombin time.

You order a mixing study, and it corrects, suggesting a factor deficiency.

You order a factor II, V, VII level, and they return with a factor II level of 19 %, factor V of 121 %, and factor VII level of 16 %. You feel the most likely cause of these numbers is vitamin K deficiency and recommend the surgical team give her vitamin K, which she receives as 5 mg IV without any untoward incident. By 48 h after you've seen her, she is improved, with better oral intake, and the prothrombin time has normalized.

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The PT Measurement

This material is covered in Chap. 1, but repeating it here will be helpful.

Historically, the PT was performed by adding tissue factor to citrated chelated, hypocalcemic plasma. The tissue factor was derived from brain tissue and acted as a thromboplastin or phospholipid complex. The tissue factor would interact with the activated factor VII in the plasma and activate the extrinsic cascade. The term extrinsic arose because the thromboplastin or tissue factor was outside of the normal flow of blood within the blood vessels. The PT also gives an indication of the activity of the clotting factors in the common pathway factors X, II and V, as well as fibrinogen.

The PT will be variably prolonged depending upon how low the factors VII, X, V, and II and fibrinogen concentrations are. This variability is different for each one of these factors. Also, the height of the PT value does not correlate linearly with the factor concentrations, so it can remain in the normal range with mildly depressed levels of some of these factors.

Up until the 1990s, different labs, in different countries, using different tissue factor sources would report out PTs in units of "seconds" to coagulate the plasma. Comparing studies on each side of the Atlantic gave disparate answers to clinical questions related to warfarin dosing so, by convention, laboratories developed the International Normalized Ratio, or INR, to standardize the PT results. A formula used by reagent companies and labs was developed.

Patient PT ^{ISI}

INR: Control PT

ISI stands for International Sensitivity Index and is calculated for each batch of tissue factor containing material. INR should only be used for monitoring warfarin (Kitchen and Preston 1999). It is not a reliable way to monitor liver function because the way it is calculated is based on plasma from patients taking warfarin.

Table 3.1 Causes of prolonged prothrombin time (PT)

Congenital
Factor VII deficiency
Acquired
Vitamin K deficiency
Coumadin use
Disseminated intravascular coagulation
Liver disease
Factor VII inhibitors

It is possible to observe a shortened PT. If the tube the blood is drawn in is not properly siliconized or if excessive activation of coagulation occurs in the tube, such as what can occur with a venipuncture that is excessively traumatic, one may have a PT that is shorter than the normal range. It should also be remembered that elevated levels of factor VII, prothrombin, and fibrinogen may lead to a thrombotic tendency, but the PT may remain in the normal range (Table 3.1).

Factor VII

Factor VII sits in a unique position in the extrinsic cascade, since it alone can activate the common pathway once it comes in contact with tissue factor.

Once one gets a consult for a prolonged PT, the next step is performing a mixing study. If there is correction of the PT by mixing a patient's plasma 1:1 with normal plasma, one knows one is dealing with a deficiency of a clotting factor, and the most likely clotting factor deficiency in this setting would be factor VII. If, on mixing, there continues to be prolongation of the PT, one may be fairly certain that there is an inhibitor to a clotting factor and factor VII inhibitors can occur, though they are rare.

Congenital factor VII deficiency is rare, as well, only occurring in 1/500,000 people (Gailani and Neff 2008). Factor VII deficiency is inherited as an autosomal recessive defect. Factor VII deficiency is usually due to an abnormally low level of factor VII function. There are some factor VII-deficient individuals who have a discordant deficiency of activity compared to antigenic levels of

factor VII (Coyer et al. 1997; Sabater-Lleal et al. 2003). Factor VII-deficient individuals can have an unpredictable story for bleeding compared with level of activity. Factor VII levels of around 20 % are adequate for hemostasis with surgery. Severe factor VII deficiency with levels <1 % is usually associated with spontaneous bleeding, like bruising, epistaxis, menometrorrhagia, and soft tissue bleeding. Some severe factor VII-deficient individuals can have hemarthroses and intracranial hemorrhage, as can be seen in factor VIII and factor IX severe deficiency. Interestingly, there are some individuals with severe factor VII deficiency with levels <1 % who have no bleeding history (Mariani et al. 2005; Girolani et al. 2007). This might be because the source of the reagent to calculate the PT and factor VII level might yield different results. Bovine and rabbit thromboplastin will give lower values than when human thromboplastin is used as a source for tissue factor in assays of factor VII activity (Roberts and Escobar 2002).

When one is called to see someone with a prolonged PT and once a mixing study demonstrates a clotting factor deficiency and the coagulation lab tells you there is a deficiency of factor VII, one must decide if this is congenital or acquired. Lifelong history of bleeding can lead one to suspect a congenital deficiency. Obtaining old lab data and finding that the PT was always prolonged also is evidence for a congenital deficiency. If one sees that old PTs were normal and now are prolonged, this is evidence for an acquired deficiency.

The most common reasons for an acquired factor VII deficiency are vitamin K deficiency (either because of warfarin use or not) and liver disease. There are some other rare problems that have been associated with factor VII deficiency, such as aplastic anemia (Weisdorf et al. 1989), homocystinuria (Dantzenberg et al. 1983), Dubin–Johnson (Seligsohn et al. 1969), and Gilbert syndrome (Seligsohn et al. 1970).

Vitamin K—The discovery of vitamin K in the 1930s by Dam and Glavind (1938) ushered in a new era of understanding of the biochemistries of blood clotting. Bleeding disease in cattle ("sweet clover disease of cattle") was found to be due to coumarols, which interfered with vitamin K dependent synthesis of prothrombin.

Vitamin K is formed by intestinal flora bacteria (Hill 1997). It is a cofactor in the posttranslational gamma-carboxylation of glutamic acids in several procoagulants, as well as for natural anticoagulants (Furie et al. 1999). Vitamin K is involved in the conversion of inactive factors II, VII, IX, and X into gamma-carboxylated forms of these procoagulants. Once gamma-carboxylated, a stable divalent anion is formed, which allows for calcium ions to bind and thus localize these clotting factors to phospholipid membranes. They also cause formation of internal calcium channels.

The vitamin K molecule undergoes chemical changes as the gamma-carboxylation process occurs. The hydroquinone is converted to an inactive vitamin K epoxide as the gamma-carboxylation takes place. Vitamin K epoxide is reduced back to an active hydroquinone by vitamin K epoxide reductase, which regenerates more vitamin K to permit further gamma-carboxylation (Ageno et al. 2012). See Fig. 3.1.

Patients may become vitamin K deficient by poor oral intake, antimicrobial use (eradicating gut flora), intestinal disorders that interfere with absorption of fat-soluble vitamins, and biliary tract disorders that lead to impaired bile acid levels entering into enterohepatic circulation. Once vitamin K levels fall, there is less gamma-carboxylation of factors II, VII, IX, and X. Because factor VII has the shortest half-life of all the clotting factors, oftentimes early vitamin K deficiency may cause an isolated elevation of the PT and not the PTT. Thus, any patient whose initial PT was normal on entering the hospital and who develops a prolonged PT in the hospital likely is vitamin K deficient.

When a patient is on warfarin, frequent consults have to do with (1) reversal of prolonged PT because of bleeding, (2) management of the warfarin during a bridging process to allow for surgery to occur, or (3) increased or decreased sensitivity of the patient to warfarin.

Giving vitamin K IV or subq can cause anaphylaxis (Fiore et al. 2001), usually to the

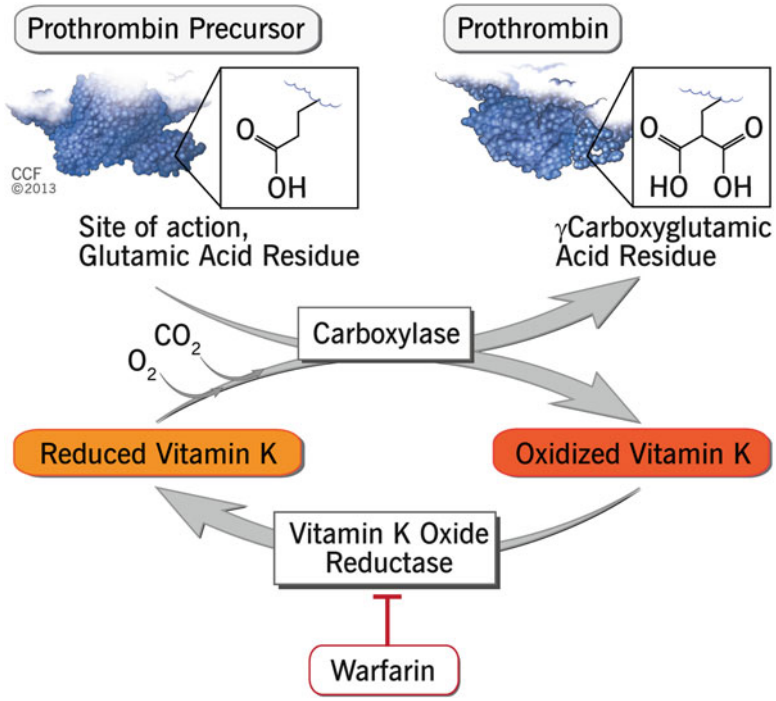

Fig. 3.1 The mechanism of "oxidized vitamin K" reduction by vitamin K oxide reductase and its inhibition by warfarin. The goal of this vitamin K cycling is the gamma-carboxylation of factors II, VII, IX, and X

cremophor part of the preparation. This is more likely to occur in elderly patients who receive >5 mg or after repeated dosing but can occur in young patients too. For IV administration, it is advised to mix the vitamin K in 100 mL of saline and administer it carefully over 20 min and to abort the infusion if any flushing or hypotension is seen (Patriquin and Crowther 2011).

When a patient has a thrombosis and is on heparin and then needs to be converted to oral warfarin, a frequent consult, as stated, is that some patients after one or two doses of warfarin suddenly have INR > 9 and then others do not budge their INR above 1, even after several days of fairly high-dose warfarin. This can be because of several different mechanisms, related to concurrent other medications, endogenous metabolism of warfarin, coincident early vitamin K deficiency, or variation in activity of the enzymes involved in vitamin K metabolism.

Pharmacogenomics and Warfarin Sensitivity or Resistance

Much has been written over the past 10 years about genetic polymorphisms that can predict how a person will metabolize warfarin, as well as other drugs (Kitzmiller et al. 2011). There are two of these with the greatest clinical relevance to warfarin metabolism: (1) cytochrome P-450 2C9, or CYP2C9, and (2) vitamin K epoxide reductase complex 1 (VKORC1).

The first of these, the hepatic cytochrome P-450 2C9, is involved in the inactivation of warfarin (Shikata et al. 2004). There are two isomers of warfarin, S-warfarin and R-warfarin. S-warfarin is the more potent of the two. S-warfarin is metabolized mostly by CYP2C9, and R-warfarin is metabolized by other cytochrome P-450 polymorphisms, mainly the CYP1A2, CYP2C19, and CYP3A4.

Those individuals with two copies of the wild-type CYP2C9 gene (CYP2C9*1) are "extensive warfarin metabolizers" and clear warfarin from the plasma very rapidly. Others, such as the allelic variants CYP2C9*2 and CYP2C9*3, may metabolize warfarin at rates up to 90 % lower (Au and Rettie 2008). Doses of warfarin to keep individuals in a specific therapeutic range may be based on these genotypes (Taube et al. 2000). Different ethnic groups also have varying genetic expression of these cytochrome P-450 variants. For example, Caucasians carry at least one copy of CYP2C9*2 eight times more frequently than African Americans, and the ratio for CYP2C9*3 is 6:1. Caucasians harbor this latter genetic allele twice as often as Asians (Garcia-Martin et al. 2006).

The other well-studied genetic variable is vitamin K 2,3-epoxide reductase (VKOR). Polymorphisms of the gene encoding the C1 subunit of VKOR, the VKORCI, affect the level of inhibition of warfarin (D'Andrea et al. 2005). These individuals with a single nucleotide polymorphism 1,639 bases upstream of VKORC1 (−1,639 G > A) need 25 % of a lower dose of warfarin than other genotypes. Groups have identified ten noncoding VKORC1 single nucleotide polymorphisms and inferred five major haplotypes. There was a low-dose haplotype group (A) and a high-dose haplotype group (B). There were significant differences between the dose of coumadin necessary to keep these different groups in a therapeutic range. Again differences in ethnicity dictated different rates of A and B haplotypes (Rieder et al. 2005).

Studies have also shown that, based on genotype, there are differences in the rapidity of rise of the PT (time to the first INR in the therapeutic ranges), time to first INR > 4, and the overall average dose of warfarin after 29 days.

Despite all the genetic information and despite the FDA approving the commercialization of kits to identify these SNPs, the American College of Chest Physicians CHEST guidelines in 2012 still do not endorse the use of genetic typing of individuals prior to initiating warfarin or for maintaining warfarin doses over a long time. The Center for Medicare and Medicaid Services (CMS) "believes that the available evidence does not demonstrate that pharmocogenomic testing of CYP2C9 or VKORC1 alleles to predict warfarin responsiveness improves health outcomes in Medicare beneficiaries". There are some provisions under which this testing might be covered, such as when the individual has had less that 5 days of warfarin therapy and if the individual is enrolled in a clinical trial examining this issue.

One setting in which knowing the genetic typing might be helpful is in determining the cause for an individual's either extreme sensitivity or extreme resistance to warfarin. When a clinician is not certain why a patient seems to need a dose of warfarin of 30 mg or more per day to nudge the INR up to 2.0 and one is suspecting that maybe the patient is just not taking his or her warfarin, knowing they harbor a SNP that dictates for resistance to warfarin may be reassuring and improve the doctor–patient relationship.

Factor VII Inhibitors

Once the consultant is called for a prolonged PT evaluation and a 1:1 mix is ordered and done and the coagulation laboratory reports the presence of an inhibitor, the consultant needs to ask the coagulation laboratory to do further testing to see if there is a specific inhibitor to factor VII. Delayed inhibition in the 1:1 mix would likely indicate a factor VII inhibited patient. These are very rare (Delmer et al. 1989; Mullighan et al. 2004). Quantifying the level of factor VII inhibitor (Bethesda titer) sometimes can guide therapy. Generally, replacing factor VII with fresh frozen plasma or recombinant VIIa may cause an anamnestic response with the development of even higher titer of inhibitor. Plasma exchange and immunosuppressants have been used in these patients with variable response.

Treatment of Factor VII Deficiency

If the 1:1 mix demonstrates a deficiency of factor VII and bleeding is occurring or surgery is being contemplated, one may choose to replace factor

VII with fresh frozen plasma, or recombinant factor VIIa may be used (Lusher et al. 1998). Factor VII levels of 5–10 % may be all that is necessary to stop bleeding. Levels of around 20 % are adequate for most surgeries.

Since factor VII has a short half-life, a single dose of 20–30 micrograms (mcg) recombinant factor VIIa/kg body weight may be enough to stop bleeding. For more significant bleeding, Factor VIIa at doses up to 20–50 mcg/kg every 2–6 h for a few days may be necessary. The doses for recombinant VIIa used in factor VIII or IX inhibitor patients who are bleeding or who need surgery may be as high as 90 mcg/kg every 2 h for several days.

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Prolonged PTT

4

Kenneth D. Friedman and Jenny H. Petkova

Introduction

Evaluation of the hemostatic mechanism of a patient is a complex undertaking. This is in part due to the complex interactions of vascular elements, platelets, and plasma coagulation proteins that subserve hemostatic mechanisms and the limitations imposed by laboratory methods. Laboratory evaluation is limited by both what can be collected into blood samples as well as the artificial test methodologies used to assess hemostatic functioning. This is more comprehensively reviewed in Chapter 1 of this text, but the basic concepts are repeated here. For evaluation of hemostasis, patient whole blood is collected into sodium citrate anticoagulant. The ratio of whole blood to anticoagulant is one part 3.2 % sodium citrate to nine parts whole blood. Platelets and some coagulation proteins are intrinsically unstable and begin degrading shortly after collection. Furthermore, laboratory platelet function is generally assessed in a hypocalcemic setting. Global assays of the coagulation mechanism, such as the prothrombin time (PT) and partial thromboplastin time (PTT), assess the cumulative output of multiple individual biochemical reactions in a platelet-poor setting. The PTT is activated by the addition of phospholipid and a negatively charged surface to activate the "contact factors" of the "intrinsic" coagulation system. The patient plasma is subsequently re-calcified, and the time between recalcification and detection of a fibrin clot is reported as the PTT in seconds. Extrapolation of results of global tests of coagulation to the clinical care of patients requires clinical judgment. The extent of coagulation factor deficiency required to prolong the PT and/or PTT varies between laboratories. For example, many PTT assays will remain normal despite reduction of factor IX level to 30 IU/dL, a level that meets the criteria for mild hemophilia B. Similarly, the level of a specific coagulation factor that is required to support normal hemostasis is not rigorously defined, but estimates have been published (Friedman and Rodgers 2009). Conversely, the PTT may be prolonged in situations not associated with hemorrhagic tendency, such as deficiency of coagulation factor XII or the presence of antibodies to phospholipid-binding proteins.

While evidence suggests that coagulation reactions occur in a coordinated fashion upon active membranes, such as activated platelets (Hoffman and Monroe 2007), it is helpful to

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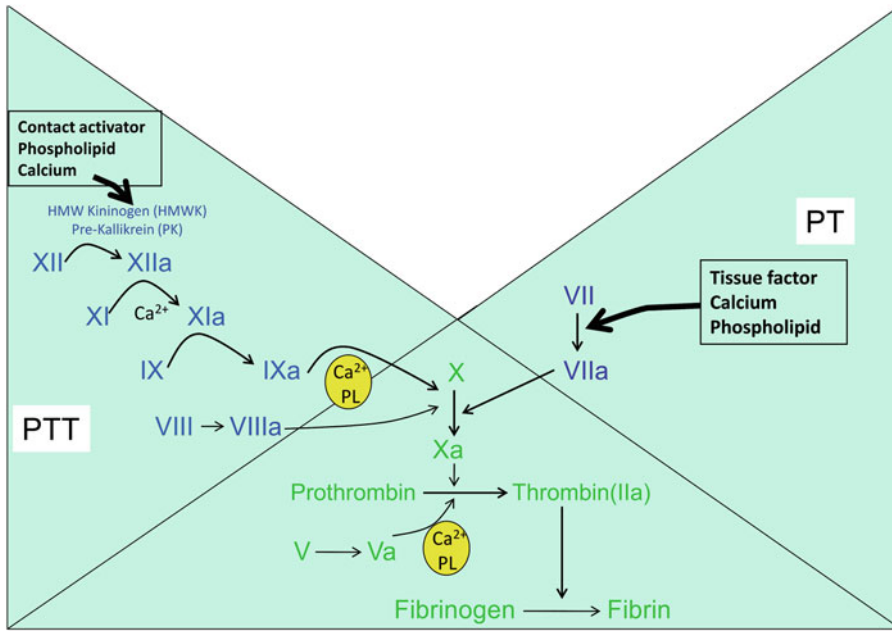

Fig. 4.1 Coagulation cascade. The factors of the "intrinsic pathway" are shown in *blue*, the factors of the "extrinsic pathway" are shown in *purple*, and the factors of the "common pathway" are shown in *green*. The PTT is performed by addition of a "contact activator" and phospholipid (PL) to plasma, followed by addition of calcium (Ca^{2+}), with reactions following the intrinsic and then the common pathways. The prothrombin time is performed by addition of tissue factor, phospholipid, and calcium to plasma, with reactions following the extrinsic and then the common pathways. Coagulation times are measured from the time of addition of calcium until the detection of fibrin clot

consider the PT and PTT assays using the traditional construct of the "coagulation cascade" as shown in Fig. 4.1. The coagulation reactions measured by the PTT and PT come together at coagulation factor X, and factors X, V, II, and fibrinogen are thus components of the "common pathway." Coagulation factors that are exclusively monitored by the PTT are known as the coagulation factors of the "intrinsic pathway." The name "intrinsic" was chosen to reflect the observation that when whole blood is collected in glass tubes it clots spontaneously due to activation of materials "intrinsic" to human blood. A subset of these are called "contact factors," factor XII, prekallikrein (PK), and high-molecular-weight kininogen (HMWK) since they get activated upon contact with negatively charged surfaces. This in turn initiates a series of reactions involving the other factors of the "intrinsic pathway" including factor XI, factor IX, and factor VIII (FVIII). This latter set of coagulation proteins is important also in propagation of a hemostatic response. Deficiency of any of the contact factors, or factors XI, IX, or VIII, may result in prolongation of the PTT. Factors IX and VIII may also play a role in tissue factor-based activation of the coagulation mechanism in vivo; however, these factors are not required to obtain a normal PT in the coagulation laboratory.

When presented with a prolonged coagulation assay, the differential diagnosis is usually between true coagulation factor deficiency and presence of a coagulation inhibitor. One approach is to request specific factor assay in an effort to isolate a deficiency. Alternatively, "mixing studies" are performed to distinguish factor deficiency from the presence of a coagulation inhibitor. Mixing studies are based upon the premise that a 50 % level of coagulation factor is sufficient to produce a normal PT or PTT. If mixing patient plasma in a 1:1 ratio with normal plasma does not produce a normal clot time, one

can conclude that the patient's plasma contains an inhibitory substance. While lupus anticoagulants are generally immediate-acting inhibitors, incubation (over 1 h) of the 1:1 mixture is frequently performed to bring out FVIII inhibitors.

Clinical Vignette 1

A 56-year-old man is noted to have an unexpectedly prolonged PTT. He visited his primary physician for evaluation of recent onset of headaches. Review of systems revealed that he has also noted occasional night sweats, pruritis after taking showers, and early satiety. Physical examination was notable for mild hypertension, plethoric facial appearance, and palpable splenomegaly without obvious hepatomegaly. Laboratory studies obtained by the patient's primary care physician revealed normal serum chemistries, with the exception of a slightly elevated uric acid; however, hematology studies showed several abnormalities as indicated below:

CBC:

WBC 12,500/μL, with normal differential (reference interval 4,000–10,000/μL)

Hematocrit 61 % (reference interval 40–51 %)

Hemoglobin 19.9 g/dL (reference interval 13.7–17.5 g/dL)

MCV 83 fL (reference interval 80–100 fL)

Platelet count 561,000/μL (reference interval 163,000–369,000/μL)

PTT 35.2 s (reference interval 21.0–28.0 s)

PT 10.2 s (reference interval 9.6 s–12.6 s)

Selected Causes

Although screening coagulation assays are generally requested for evaluation of either hemorrhagic or thrombotic conditions, screening coagulation studies are occasionally ordered in evaluation of other medical conditions. In addition to consideration of pathologic conditions, interpretation of a prolonged PTT in a patient without clinical symptoms of a hemostatic defect should raise a question concerning the integrity of the assay result. As indicated in Table 4.1, there are several situations under which spurious abnormalities should be considered.

Table 4.1 Causes of an isolated prolonged PTT

A. Physiologic and artifactual causes
1. Normal neonate or infant
2. Erythrocytosis
3. Improper sample handling
a. "Short fill" of citrate tube
b. Delayed separation of plasma
B. Coagulation factor deficiency
1. Deficiency of a contact factor
a. Factor XII
b. Prekallikrein
c. High-molecular-weight kininogen
2. Deficiency of a propagation-phase factor
a. Factor VIII (hemophilia A and von Willebrand disease)
b. Factor IX (hemophilia B)
c. Factor XI
C. Coagulation factor inhibition/clearance
1. Inhibitors associated with bleeding
a. Factor VIII inhibitor
b. Acquired von Willebrand disease
2. Lupus anticoagulant
D. Presence of an anticoagulant drug
1. Unfractionated heparin
2. Low-molecular-weight heparin
3. Direct thrombin inhibitors

Neonates and Infants

While the reference interval for PTT varies considerably between laboratories (as a consequence of the use of varying reagents), the PTT of newborns and infants is predictably prolonged compared to an adult reference interval. This is primarily due to low levels of "contact factors" during early childhood. Several of the vitamin K-dependent coagulation factors are also reduced compared to adult levels, while levels of factors V, VIII, and fibrinogen are similar to those observed in adults (Flanders et al. 2006; Monagle et al. 2006). Abnormalities observed in young infants should be reassessed after 6 months of age, because most coagulation proteins rise towards the adult range by approximately that time (Tcherniantchouk et al. 2013); however, a notable exception is protein C.

Erythrocytosis

Citrate anticoagulant is used when collecting samples for coagulation testing. During performance of the PTT, a fixed concentration of calcium is added back to the sample to exceed the chelating capacity of citrate and allow coagulation to proceed. In the event of a short draw or in the setting of marked erythrocytosis, an insufficient volume of plasma is collected, resulting in under-dilution of the citrate anticoagulant. The calcium added in the laboratory may then be insufficient to overcome the citrate effect, resulting in an artifactually prolonged PTT. Adjustment of anticoagulant in the collection system has been recommended for hematocrit over 55 % (Adcock et al. 2008).

Improper Sample Handling

Delays in sample processing or inappropriate storage of blood may lead to falsely elevated PTT results. Storage of whole blood samples in ice has been demonstrated to result in reduced levels of FVIII and von Willebrand factor (VWF). In addition, both factors VIII and V are "labile factors," and levels will decline in plasma samples maintained in an unfrozen state (Favaloro et al. 2004). It is recommended that samples collected for coagulation assay be separated to plasma within 4 h of collection and be either tested within that interval or frozen for subsequent analysis (Adcock et al. 2008).

Sample Contaminated with Anticoagulant

Contamination of blood samples with anticoagulant is a common problem encountered in hospitalized patients. This problem is especially common in patients being managed in intensive care settings where heparin may be utilized to keep lines open (Carcao and Blanchette 2010). Heparin binds antithrombin and accelerates the inactivation of thrombin, factor Xa, and to a lesser extent FIXa, FXIa, and FXIIa. A high concentration of heparin will prolong both the PTT and the PT; at lower concentrations an isolated prolongation of the PTT will be observed. Heparin contamination can be confirmed by assessment of the thrombin time or by measurement of anti-Xa assay (Tcherniantchouk et al. 2013). Medical history will disclose if a patient is receiving therapeutic anticoagulant medication. Oral dabigatran and intravenous argatroban, bivalirudin, and lepirudin (direct thrombin inhibitors) prolong the PTT (Lindahl et al. 2011; van Ryn et al. 2010; DiNisio et al. 2005), while anticoagulants directed at factor Xa (such as low-molecular-weight heparin, fondaparinux, and rivaroxaban) are less likely to affect this coagulation assay (Hillarp et al. 2011).

Diagnostic Approach

Evaluation of an unexpectedly prolonged PTT begins by reviewing the indication for sending the assay and consideration of the specifics of sample management. In an outpatient setting, contamination of a clinical sample with anticoagulant is unlikely. In an outpatient office setting in which samples are collected and then stored for subsequent analysis by a referral laboratory, artifacts may occur due to either cold storage of whole blood or delayed sample processing. Finally, the situation of the patient needs to be considered. In this particular case, the patient has been demonstrated to have marked erythrocytosis. Settings in which marked erythrocytosis occurs include the newborn state, marked dehydration, chronic cardiopulmonary disease, hypoventilation syndromes, myeloproliferative neoplasm, some hemoglobinopathies, and syndromes in which excessive erythropoietin is being synthesized. In this case, the clinical and laboratory parameters are suggestive of a polycythemia vera, a myeloproliferative neoplasm.

Clinical Vignette 2

A 19-year-old college student presents to the emergency room with a painful and bruised left thigh. He had been "elbowed" while playing intramural basketball 1 day earlier. Overnight, his thigh became progressively swollen, discolored, and painful despite his applying cold compresses and his taking ibuprofen. His personal medical history is otherwise only remarkable except for requiring a revisit to his dentist because

(continued)

of bleeding that occurred on the evening after extraction of wisdom teeth. His only other surgical intervention was circumcision as a newborn; he was not aware of any complications of that procedure. Family history is noteworthy for an uncle who had a bleeding complication after cholecystectomy that resulted in prolonged hospitalization. Physical exam confirmed the presence of painfully enlarged left thigh with ecchymosis. In addition the patient appeared to have a small resolving hematoma on the anterior tibial surface of that leg. When asked about this, he indicated that he knocked into a box at school several days earlier and that he commonly develops such bruises on his legs if he hits something.

Laboratory data of note include the following:

CBC:

WBC 8,500/μL, with normal differential (reference interval 4,000–10,000/μL)

Hematocrit 43 % (reference interval 40–51 %)

Hemoglobin 14.2 g/dL (reference interval 13.7–17.5 g/dL)

MCV 89 fL (reference interval 80–100 fL)

Platelet count 341,000/μL (reference interval 163,000–369,000/μL)

PTT 34.2 s (reference interval 21.0–28.0 s)

PT 10.2 s (reference interval 9.6 s–12.6 s)

Deficiency of Contact Factors

During the performance of the PTT, coagulation is initiated by contact activation. Deficiency of contact factors is not associated with hemorrhagic tendency, and patients typically present with an unexpectedly prolonged PTT with no bleeding history. An autosomal recessive trait, congenital factor XII deficiency is not uncommon, being identified in 1.5–3.0 % of healthy blood donors (Halbmayer et al. 1994). Other components of the contact system include PK and HMWK. Deficiency of these factors is very uncommon. Data suggest that HMWK functions to bind PK and FXI to anionic surfaces, accelerating their activation by surface-bound factor XIIa. While deficiency of PK may be inferred by observing normalization of the PTT upon prolonged incubation with particulate activators, that is not true of HMWK deficiency (Abildgaard and Harrison 1974). Quantitative assays of PK and HMWK are typically only available from reference laboratories (Tcherniantchouk et al. 2013).

Deficiency of Factors Involved in Propagation Phase of the Coagulation

Hemophilia A and B

Hemophilia A, also known as classical hemophilia, results from congenital deficiency of FVIII. This is an X-linked recessive condition, with a similar prevalence of approximately 11 per 100,000 among the various racial groups present within the United States (Soucie et al. 1998). Upon release into circulation, FVIII is non-covalently bound by VWF which prevents premature degradation of FVIII and serves to co-localize FVIII at sites of vascular injury. During hemostatic events, tissue factor interacts with activated factor VIIa (FVIIa), which in turn interacts and activates both factors IX and X, with eventual activation of prothrombin to thrombin. Through a positive feedback mechanism, thrombin cleaves factors XI, VIII, and V to produce factors XIa, VIIIa, and Va. In turn, factor XIa drives the "propagation phase" of hemostasis, generating a large amount to thrombin through the serial actions of "tenase" (a complex containing factors IXa and VIIIa) and "prothrombinase" (a complex containing factors Xa and Va) complexes. Generation of these larger quantities of thrombin allows thicker fibrin strand formation from fibrinogen, activation of factor XIII to cross-link fibrin strands, and activation of thrombin-activable fibrinolysis inhibitor (TAFI) to down regulate fibrinolysis. Patients with hemophilia A and hemophilia B have poor hemostatic function as a consequence of failure to generate sufficient thrombin through the propagation phase of hemostasis.

The severity of symptoms in hemophilia A is related to the degree of severity of coagulation factor deficiency. Three levels of severity of hemophilia A have been defined by a consensus committee (White et al. 2001). Severe deficiency (less than 1 IU/dL) is generally manifested by "spontaneous" bleeding, usually into joints and muscles. Without treatment, these patients typically progressed to develop severe and debilitating chronic arthropathy. Moderate hemophilia (defined with factor levels between 1 and 5 IU/dL) is associated with less frequent and less severe hemarthrosis. Patients with mild hemophilia (defined by factor levels of 6–40 IU/dL) seldom have unexplained bleeding but typically present with excessive bleeding after trauma or with surgery. Hemophilia A arises due to genetic defects in the FVIII gene that is carried on the X-chromosome. New mutations of the FVIII gene are not unusual, and as many as one-third of cases of hemophilia A arise from new mutations. In such situations, there is no antecedent family history of hemophilia. Female carriers of hemophilia A may have low levels of FVIII as a consequence of skewed X-chromosome inactivation; symptomatic carriers may experience menorrhagia, oral bleeding, and excessive bleeding with trauma or childbirth.

Hemophilia B due to congenital deficiency of factor IX is 4–8 times less common than hemophilia A but is clinically indistinguishable from congenital FVIII deficiency. Both disorders result in bleeding related to insufficient generation of thrombin at sites of vascular injury, both are inherited in an X-linked fashion, and like hemophilia A, the severity of disease is graded as severe, moderate, or mild using the same factor level criteria. However, unlike hemophilia A in which spontaneous new mutations are common, the rate of new mutation of the factor IX gene is low, such that most patients with hemophilia B do have a positive family history.

Diagnosis of hemophilia is based on a clinical history of bleeding or suspicion based on history of an affected male family member. Isolated prolongation of the PTT which corrects on mixing with normal plasma is generally observed. However, the sensitivity of PTT reagents to deficiency of factor VIII or IX varies, such that if there is sufficient clinical suspicion of mild hemophilia, specific assay of factor VIII or IX level is indicated despite a normal PTT. Several caveats related to diagnosis of hemophilia A and B should be kept in mind. First, as indicated above, FVIII levels may be artifactually low in samples that are not handled properly (Favaloro et al. 2004). In addition, FVIII is carried in plasma in complex with VWF. As a consequence, FVIII levels may be low on the basis of von Willebrand disease (VWD). In patients with severe deficiency of VWF (type 3 VWD) or patient with the rare variant associated with deficient binding of FVIII by VWF (type 2N VWD), levels of FVIII are typically in the range observed in moderate to mild hemophilia A (Nichols et al. 2008). In a patient without a family history suggestive of sex-linked inheritance of hemophilia A or in a patient with unexpectedly short survival of infused replacement FVIII, assessment of VWF function is indicated. Finally, factor IX levels are low during the initial 6 months of life; repeat study is indicated to confirm a diagnosis of moderate or mild hemophilia B, if that diagnosis was made in a young child without a positive family history.

Treatment of bleeding in hemophilia patients is based upon the specific diagnosis (hemophilia A versus hemophilia B, the severity of factor deficiency) and the severity of the hemostatic challenge (Dunn and Abshire 2004). Management of these patients is best accomplished in collaboration with an experienced hematologist. The mainstay of hemophilia care is coagulation factor replacement. Concentrates are either plasma derived or generated by recombinant technology. Infusions can be delivered in a prophylactic fashion for patients with severe hemophilia or a patient anticipating a hemostatic challenge such as surgery. Alternatively, replacement therapy may be performed episodically in response to a hemorrhagic episode. Because of the need for urgent care of joint and muscle bleeds, many patients with moderate and severe hemophilia store factor products at home and are educated in the techniques of IV self-infusion.

The dose and frequency of factor infusion are calculated based upon the desired increment in factor level required by the patient's hemostatic challenge, half-life of the product (typically 8–12 h for FVIII and 18–24 h for factor IX), and

empiric recovery data. For FVIII, infusion of 1 U/Kg will typically raise the FVIII level by 2 IU/dL. For plasma-derived factor IX the rise is typically 1 IU/dL, while recombinant factor IX has a recovery that is approximately 30 % lower than that of the plasma-derived factor. For life-threatening bleeds, it is appropriate to replace FVIII or factor IX level up to near 100 IU/dL. Tables have been published which provide guidance for the desired factor level and duration of replacement for various situations such as spontaneous joint bleeds, muscle bleeds, and surgery (Dunn and Abshire 2004). A significant complication of factor replacement therapy is the development of inhibitory antibody. This complication is most common in patients with severe hemophilia A, affecting up to 15 % of those patients. Inhibitor development should be clinically suspected when a previously treated patient fails to show symptomatic response to factor replacement therapy, and is confirmed by measurement of inhibitor titer. Confirmation of the absence of an inhibitor is recommended in the preoperative evaluation of severe hemophilia patients anticipating surgery. Treatment of inhibitor patients is complex. Inhibitors may be eradicated with immune tolerance induction therapy (ITT) involving repeated daily exposure to factor concentrate (Dimichele 2007a).

Desmopressin (DDAVP) induces release of FVIII/VWF complex from endothelial cells and has been effective in treating minor bleeds in patients with mild hemophilia A. DDAVP generally raises FVIII levels in these patients two- to threefold, but confirmation of responsiveness is recommended before using this approach to treat minor bleeds. DDAVP treatment is associated with fluid retention, and patients should be counseled to restrict fluid intake after its use to avoid hyponatremia (Mannucci 1998). Antifibrinolytic agents such as aminocaproic acid or tranexamic acid may also have a role in the treatment of mucosal bleeding (Mannucci 1998).

Factor XI Deficiency

The clinical manifestations of factor XI deficiency are quite variable and tend to be less severe than those observed in hemophilia A or B. Oral, nasal, and genitourinary bleeding is more frequently described, probably related to increased fibrinolytic activity in these areas. Spontaneous bleeding is rare, but excessive bleeding with trauma is not uncommon. Factor XI deficiency is transmitted as an incompletely autosomal recessive trait, with levels <20 IU/dL observed in homozygotes and levels of 35–65 IU/dL reported in heterozygote carriers. This disorder is more often found in people of Ashkenazi Jewish heritage, in whom up to 0.3 % are homozygotes (Seligsohn 1978). The PTT is generally prolonged in patients with homozygous defects. Since PTT is inconsistently prolonged in heterozygous factor XI deficiency, specific factor assay is suggested for patients where this diagnosis is in question. Factor XI levels do not reliably predict bleeding tendency. Mucosal bleeding can often be managed with antifibrinolytic therapy alone. However, for a patient with a history of bleeding, invasive surgery is often managed with a combination of antifibrinolytics and factor replacement. In many countries, purified factor XI products are unavailable (including the United States), and plasma infusion is the only replacement product available. Depending on the hemostatic challenge, target levels of 30–50 IU/dL are suggested. Inhibitor formation may complicate homozygous factor XI deficiency (Gomez and Bolton-Maggs 2008).

Von Willebrand Disease

VWD is a bleeding disorder characterized by deficiency or dysfunction of VWF. VWF is a large multimer protein produced by endothelial cells and megakaryocytes, with two distinct roles in the hemostatic mechanism. It subserves platelet adhesion at sites of vascular injury, and it serves as a chaperone for FVIII. Owing to its multiple functions and multimer nature, VWD has been classified into three main categories (see Table 4.2). Type 1 VWD is the most common form of VWD, reported to be present in up to 1 % of the population. It is a variably penetrant autosomal dominant disorder characterized by mucosal bleeding and quantitative deficiency of VWF. Type 3 VWD is a rare autosomal recessive condition characterized by virtual absence of VWF from the plasma. As a consequence of

absent chaperone function, FVIII levels are also reduced in these patients. Finally, type 2 VWD represents approximately 15–20 % of cases and is characterized by qualitative defects of VWF function. Type 2 VWD is further subclassified into five categories, based on the nature of the defects. All but type 2N are inherited as autosomal dominant traits. Type 2A VWD accounts for the majority of type 2 disease and is characterized by reduced VWF activity levels, absence of the larger molecular weight multimers from plasma, and reduced support of platelet adhesion due to the absence of large multimers. Type 2M VWD is a rare disorder, resulting from mutations that specifically undermine the interaction of VWF with platelets, but the multimer distribution is normal. Type 2B and platelet-type VWD result from increased interaction of VWF with platelets, resulting in depletion of both the larger VWF multimers and platelets from circulation. Finally, type 2N VWD results from mutations that undermine the chaperone function of VWF, producing an autosomal recessive form of FVIII deficiency. Diagnosis of type 2N VWD is important, as the genetics and therapy differ from those of mild hemophilia A (Nichols et al. 2008). In addition to these congenital defects, acquired forms of VWD have also been described and are further discussed below.

Diagnosis of VWD begins with documenting a history of bleeding, exploring the family history, and then performing a careful laboratory assessment. Laboratory screening begins with checking the platelet count, PT, and PTT. The PTT may not be prolonged if the FVIII level is over 40 IU/dL, and PFA-100 testing is not recommended, as it is relatively insensitive to milder forms of VWD (Nichols et al. 2008; Quiroga et al. 2004). To screen effectively for VWD, three tests are suggested: VWF antigen (quantifying the protein concentration), VWF ristocetin cofactor activity (measuring the functional interactivity of VWF), and FVIII activity. Bleeding symptoms are generally not attributable to VWD if all three quantitative studies are over 50 IU/dL (Nichols et al. 2008). However, one should keep in mind that VWF is an acute-phase reactant, and if type 1 VWD remains a clinical concern after initial testing, repeated VWF studies may be informative. Diagnosis of VWD requires an integrated analysis of the history and results of screening tests, evaluation of VWF levels for quantitative deficiency, and then further evaluation of the ratio of VWF ristocetin cofactor activity or FVIII activity to the VWF antigen to decide whether type 2 VWD is a clinical possibility. Further studies are required in order to characterize type 2 variants (see Table 4.2).

Management of VWD is multifaceted (Gill 2004). Minor bleeding symptoms may require no intervention. Menorrhagia is frequently managed with hormonal therapy (e.g., oral contraceptives). Desmopressin is considered primary therapy for type 1 VWD, given the same caveats listed above for mild hemophilia A (Mannucci 2004). Some type 2A and type 2N patients will have a sufficient (but transient) response to DDAVP to allow treatment of minor bleeds. However, DDAVP is relatively contraindicated in type 2B and platelet-type disease, as DDAVP may exacerbate thrombocytopenia in those two variants. DDAVP is ineffective in type 3 VWD. For major bleeds or surgery, and in patients who are unresponsive to DDAVP, replacement therapy with exogenous VWF is required. The preferred replacement product is an intermediate-purity VWF-containing plasma-derived antihemophilic factor concentrate (Federici and Mannucci 2007). Therapy of acquired VWD varies with the underlying clinical condition and severity of hemostatic challenge (see discussion below).

Diagnostic Approach

Clinical Vignette 2 presents a young man with a history of bruising, an excessively bruised leg after trauma, and a prolonged PTT. History suggests that there might be another family member who had bleeding manifestations. There is value in taking a careful history of hemostatic challenges such as dental extractions and surgical procedures to define the duration of bleeding tendency. Similarly, careful family history may indicate that a bleeding tendency is inherited as a sex-linked male trait or in an autosomal dominant pattern. In this case, confirming that the uncle with bleeding was related through the patient's

Table 4.2 von Willebrand disease variants

	Normal	Type 1	Type 3	Type 2A	Type 2B	Type 2M	Type 2N	PT-VWD
VWF:Ag	N	↓	Absent	↓	↓	N or ↓	N or ↓	↓
VWF:RCo	N	↓	Absent	↓↓↓	↓↓	↓	N or ↓	↓↓
FVIII	N	↓ or N	↓↓	N or ↓	N or ↓	N	↓↓	N or ↓
Multimer	N	N	Absent	Abnormal	Abnormal	N	N	Abnormal
LD-RIPA	Absent	Absent	Absent	Absent	↑↑	Absent	Absent	↑↑
Platelet count	N	N	N	N	↓ (rarely N)	N	N	↓
Response to DDAVP		Good	None	Poor	Decreases platelets	Poor	Poor	Decreases platelets
Usual treatment		DDAVP VWF Conc	VWF Conc	VWF Conc (DDAVP)	VWF Conc	VWF Conc (DDAVP)	VWF Conc (DDAVP)	Platelet VWF Conc

Summary of von Willebrand disease types: von Willebrand antigen (VWF:Ag), von Willebrand ristocetin cofactor activity (VWF:RCo), factor VIII activity (FVIII), VWF multimer distribution (multimer), low-dose ristocetin-induced platelet aggregation (LD-RIPA). Treatments are desmopressin (DDAVP), VWF-containing concentrates (VWF conc), or platelet transfusion (platelets)

mother would suggest sex-linked inheritance, as occurs with hemophilia A and hemophilia B. Next steps might be PTT with mixing study to exclude an inhibitor or just proceeding to factor assay and von Willebrand studies in this patient with a long-term history of bruising symptoms. If mild factor IX deficiency is uncovered, the laboratory evaluation might end. However, if mild FVIII deficiency is uncovered and VWF levels are normal, it would be reasonable to perform studies to exclude type 2N VWD before concluding that the patient's diagnosis is mild hemophilia A. Indeed, clinical vignette 2's FVIII:C level was 7 %. All other clotting factor assays performed were normal.

Clinical Vignette 3

An 82-year man presents to the emergency room with an expanding hematoma on his right arm. He had noted increased bruising over the previous 2 weeks but became alarmed when he developed a bruise on his lower right arm that progressed to a tight feeling accompanied by numbness of several fingers. He has been previously remarkably healthy with exceptions of well-controlled hypertension and localized prostate carcinoma that was treated with external beam radiation therapy. He indicated that he has never had any bleeding issues and that there were no complications associated with the multiple prostate biopsies that were collected to diagnose his prostate tumor. Current medications include aspirin 81 mg/day that he has taken for several years and tamsulosin to optimize urinary flow. Physical exam reveals bruises on all four extremities in various stages of evolution, tight distention of his lower right arm, decreased pulse at the right radial location, somewhat swollen and cyanotic fingers, and decreased sensation in his fingers.

Laboratory data include the following:

CBC:

WBC 9,400/μL, with normal differential (reference interval 4,000–10,000/μL)

Hematocrit 39 % (reference interval 40–51 %)

Hemoglobin 12.8 g/dL (reference interval 13.7–17.5 g/dL)

MCV 89 fL (reference interval 80–100 fL)

Platelet count 241,000/μL (reference interval 163,000–369,000/μL)

PTT 72.2 s (reference interval 21.0–28.0 s)

PT 10.2 s (reference interval 9.6 s–12.6 s)

Fibrinogen 321 mg/dL (reference interval 236–448 mg/dL)

D-Dimer 1.3 FEU (reference interval < 0.6 FEU)

Selected Causes

Acquired coagulation factor inhibitors are autoantibodies directed against a coagulation factor in a person without an underlying bleeding disorder. These rare hemorrhagic disorders present with recent onset of bleeding and may be life threatening. While autoantibody to factor V and factor II are associated with prolongation of the PT and to a lesser degree the PTT, antibody to either factor VIII or VWF will present with an isolated prolongation of the PTT.

Autoantibody to Factor VIII

The incidence of FVIII inhibitory autoantibodies has a bimodal distribution, with a small peak in younger individuals, primarily postpartum, and then a major peak of up to 14.7 per million in older individuals aged 60–80 years (Collins 2011). FVIII inhibitors are frequently associated with disease states and are thought to arise from dysregulation of immune function. Disease associations include autoimmune disorders such as systemic lupus erythematosis, rheumatoid arthritis, and malignancies (especially lymphoproliferative disorders) but may occasionally occur as a consequence of pregnancy. The severity of bleeding symptoms is variable, but often it is quite severe. In contrast to congenital FVIII deficiency, patients with autoimmune inhibitors generally present with mucocutaneous bleeding (such as epistaxis or GI bleeding) or soft tissue bleeding, while joint and muscle bleeds are com-

paratively rare. Laboratory diagnosis is similar to that of congenital hemophilia, starting with observation of a prolonged PTT with normal PT. The PTT may correct on immediate mix with normal plasma but would be expected to be prolonged if the mix is incubated. Clinical presentation is critical for distinction of FVIII inhibitor from a lupus anticoagulant. Except in rare instances of concomitant autoantibody to factor II, lupus inhibitors are not associated with hemorrhagic tendency. FVIII inhibitor can be further documented by specific assay of the FVIII level and measurement of FVIII inhibitor by Bethesda assay. Interestingly, due to unusual kinetics of FVIII autoantibody, a low but detectable residual FVIII level may be observed in patients with this condition. Management of autoantibody to FVIII is twofold. For treatment of bleeding in patients with acquired inhibitors (and also inhibitor patients with congenital deficiency of FVIII), FVIII replacement may be successful in patients with low-level inhibitors (<5 BU); owing to the time-dependent nature of inhibition, FVIII infusion may be successful. For higher titer inhibitors, FVIII bypassing agents such as recombinant FVIIa or activated prothrombin complex concentrate may be required (Collins 2011; Hay et al. 2006). Because of the inability to predict who might have a fatal hemorrhage, immune suppression should also be considered in patients with autoantibodies. Therapies have included corticosteroids and cyclophosphamide and more recently rituximab.

Autoantibody inhibitors are rarely directed against the other coagulation factors involved in the intrinsic pathway, but such cases have been described. Similar to what is observed with hemophilia A, occasionally patients with congenital deficiency of factor IX or factor XI will develop inhibitory antibodies to these factors (Salomon et al. 2006; Dimichele 2007b).

Autoantibody to VWF

The incidence of acquired von Willebrand disease (AVWD) has not been established. The ISTH registry documents that lymphoproliferative (48 %), cardiovascular (21 %), myeloproliferative (15 %), other neoplastic (5 %), and autoimmune disorders (2 %) are the most common associated conditions (Tiede et al. 2011). While autoantibody may contribute to the pathogenesis of this condition in patients with lymphoproliferative disorders, sequestration of VWF may be an important mechanism in patients with myeloma and myeloproliferative neoplasm, while shear-induced degradation of VWF contributes to loss of VWF function in patients with aortic stenosis and LVAD devices. AVWS should be considered in a patient with an acquired bleeding disorder in whom the laboratory findings are consistent with VWD. In addition to the typical studies suggested for the evaluation of congenital VWD, serum protein electrophoresis to screen for monoclonal gammopathy and VWF propeptide assay may be informative in the evaluation of AVWD. Treatment of AVWD varies depending on the clinical situation. In addition to therapy aimed at an underlying condition, treatment may include replacement therapy via either DDAVP- or VWF-containing concentrate, bypassing agents such as recombinant FVIIa or antifibrinolytics. Intravenous immunoglobulin appears to have been useful in patients with monoclonal gammopathy of unknown significance (Tiede et al. 2011).

Diagnostic Approach for Clinical Vignette 3

This is an older individual with a history consistent with an acquired coagulation disorder. Coagulation profile shows an elevated PTT. The fibrinogen and platelet counts are normal; D-dimer may be elevated due to resolving hematomas. Given his age and history, acquired coagulation inhibitor should be suspected. Evaluation would include 1:1 mix of the PTT, but given the bleeding phenotype, request for FVIII assay should be ordered concomitantly. In this case, the PTT on a non-incubated 1:1 mix was prolonged at 56 s and the FVIII level was reported at 6 IU/dL. Observing some residual FVIII activity is not unusual in patients with acquired FVIII inhibitor. A FVIII inhibitor by Bethesda assay was then requested and was reported as “nonparallel to the standard curve; however, the dilution of plasma giving the residual FVIII level closest to 50 % suggests assignment of the result at 16 BU.” Observing nonparallel kinetics is not unusual for

acquired autoantibody, a situation that is rarely seen in patients with congenital hemophilia A who develop inhibitors. Therefore, this patient did, indeed, have a FVIII inhibitor.

Clinical Vignette 4

A 37-year-old woman is referred for evaluation of coagulopathy and thrombosis. Her medical history is remarkable only for an uncomplicated first pregnancy at age 32 years, but a second-trimester spontaneous fetal demise complicated her second pregnancy at age 34 years. Recently, at age 37 years, she developed an unprovoked deep vein thrombosis and was placed on low-molecular-weight heparin and warfarin through an urgent care clinic. A week later, her INR was 2.4, and the low-molecular-weight heparin was discontinued. She has no family history of thrombotic illness. At the current time, her only medication is warfarin, 5 mg/day. Laboratory results before anticoagulation and now 2 weeks later were as follows:

At the urgent care clinic CBC:

WBC 6,400/μL, with normal differential (reference interval 4,000–10,000/μL)

Hematocrit 39 % (reference interval 34–45 %)

Hemoglobin 12.8 g/dL (reference interval 11.2–15.7 g/dL)

MCV 89 fL (reference interval 80–100 fL)

Platelet count 114,000/μL (reference interval 163,000–369,000/μL)

PTT 58.2 s (reference interval 21.0–28.0 s)

PT 11.2 s (reference interval 9.6 s–12.6 s)

INR 1.0

Two weeks after initiation of warfarin:

Repeated platelet count is 109,000 (reference interval 163,000–369,000/μL)

PTT 68.2 s (reference interval 21.0–28.0 s)

PT 26.6 s (reference interval 9.6 s–12.6 s)

INR 2.4

Selected Causes

Antiphospholipid Syndrome

A diagnosis of antiphospholipid syndrome (APS) is based on clinical criteria (vascular thrombosis or specific pregnancy morbidity) and laboratory evidence of persistent presence of antiphospholipid antibodies (Miyakis et al. 2006). Thrombosis may be arterial or venous. Complications of pregnancy include an in utero fetal demise after 10 weeks gestation; premature birth of a morphologically normal child before 34 weeks gestation in which the pregnancy was complicated by preeclampsia, eclampsia, or placental insufficiency; or more than two unexplained consecutive pregnancy losses before 10 weeks gestation (see Pregnancy chapter). Antiphospholipid antibodies include a fairly heterogeneous set of laboratory findings, but the criteria for diagnosis of APS have focused on demonstrating the presence of a lupus anticoagulant (Pengo et al. 2009), anticardiolipin (ACA) IgG or IgM over 40 GPL or MPL, or presence of anti-β2-glycoprotein 1 (anti-B2GP1) IgG or IgM over the 99th percentile (Miyakis et al. 2006). While these criteria were designed to facilitate clinical studies, they have generally been used for individual patient identification. A diagnosis of APS is usually reserved for patients who meet both the clinical and laboratory criteria and in whom an alternative explanation for their medical situation is lacking. Other clinical characteristics associated with APS include thrombocytopenia, livedo reticularis, valvular heart lesions, and nephropathy. Laboratory measurement of antiphospholipid antibodies has limitations. It is recommended that if APS is suspected both lupus anticoagulants and serologic testing for ACA and anti-B2GP1 antibody be performed. Positive studies should be repeated in 12 weeks to show persistence. Lupus anticoagulants (LA) are identified by their ability to prolong phospholipid-dependent coagulation assays in vitro (such as the PTT or the dilute Russell viper venom time (DRVVT)), failure of the clot time to correct upon 1:1 mix, and improvement of the clot time upon increasing the phospholipid concentration in the assay. Because of the heterogeneity of LA,

it is recommended that two phospholipid clot times are used to screen for an LA and that specific coagulation factor inhibitors are excluded either by clinical criteria or by laboratory assay. Diagnosis of LA is complicated when patients are already on treatment with anticoagulant medication. ACA and anti-B2GP1 are quantified by ELISA methods. Furthermore, a diagnosis of APS may be difficult in thrombocytopenic patients. In that setting, alternative diagnoses such as disseminated intravascular coagulopathy (DIC), thrombotic thrombocytopenic purpura (TTP), heparin-associated thrombocytopenia (HIT), and unusual syndromes such as paroxysmal nocturnal hemoglobinuria (PNH) may all have to be considered and excluded with careful clinical and laboratory assessments (Lim 2009).

The pathogenesis of clinical complications of APS remains to be fully elucidated. The most common site for venous thrombosis is the deep leg veins, but unusual locations include axillary, cerebral, and splanchnic vessels. Arterial thrombosis often presents as cerebral events, but any arterial bed is at risk. Thrombotic complications are generally treated with anticoagulant medication, but some data supports the use of aspirin in patients whose clinical manifestation was stroke. Pregnancy outcomes in women with recurrent pregnancy loss have been improved through administration of heparin and aspirin (Lim 2009).

Diagnostic Approach for Clinical Vignette 4

At the time of presentation with a deep vein thrombosis, one must take a careful history to exclude provoked thrombotic events related to acute illness or trauma, pregnancy or hormonal therapy, chronic inflammatory or infectious disease, myeloproliferative disorder, neoplasm, or rare disorders such as paroxysmal nocturnal hemoglobinurea. The patient described in clinical vignette 4 had a prolonged PTT at presentation of her thrombosis, suggesting either consumptive coagulopathy due to systemic disease, tumor, or extensive thrombus formation, liver disease, or APS. At the time of her initial presentation to the urgent care facility, history and physical findings probably were undertaken to exclude many underlying problems, and normal WBC differential argues against an obvious marrow disorder. The normal PT argues against liver disease, and occult malignancy presenting as DIC with thrombosis would be unusual in a woman of this age. The history of a late pregnancy loss, long PTT, thrombocytopenia, and thrombotic event raise a question of APS. On warfarin, the platelet count has been stable and the PTT is more prolonged. Thrombocytopenia is not expected with inherited prothrombotic states such as deficiency of antithrombin, protein C, or protein S, but it is a component of acquired conditions such as APS. While a target INR of 2–3 is sufficient for warfarin anticoagulation in APS (Crowther et al. 2003), there appears to be an increased incidence of recurrent thrombosis in this setting. Serologic assay for APS can be performed while on warfarin, but studies for lupus anticoagulant need to be interpreted cautiously in that setting.

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Prolongation of Both PT and aPTT

5

Bernard J. Silver

The coagulation cascade is typically assessed by measurement of the prothrombin time (PT) and activated partial thromboplastin time (aPTT). While these are crude screening tests for abnormalities of coagulation, they do provide a rapid way of detecting significant deficiencies in the extrinsic, intrinsic, contact, and common pathways of coagulation which may or may not reflect an increased risk of bleeding. Unlike the already described isolated elevations in either the PT or the aPTT (Chaps. 3 and 4), a finding of prolongation of both clotting times implies a defect in the more distal or common pathway of the coagulation cascade (Fig. 5.1).

Inherited single or combined defects in this group of coagulation reactions are among the rarest coagulation disorders. Both inherited and acquired defects discussed in this chapter are listed in Table 5.1.

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Congenital Fibrinogen Disorders

Clinical Vignette 1

A 52-year-old female with a PMH of diabetes, osteoarthritis, and migraine headaches referred for preoperative evaluation of prolonged clotting times. A total knee replacement was scheduled. She had had previous minor procedures (endoscopy, skin biopsy) but no major surgery. She had recurrent epistaxis as a child but no menorrhagia or any other type of excessive bleeding. There was no history of thrombosis or liver disease. Her laboratory testing revealed the following:

PT 16.5 (9–13 s)
1:1 mix with normal plasma 12.0 s
aPTT 33.2 (24.4–31.7 s)
Fibrinogen clot 66 (200–400 mg/dL)
Fibrinogen antigen 352 (149–353 mg/dL)
Thrombin time 36.2 (15.1–18.5 s)
Reptilase time >60 (18–22 s)
Factors II, V, VII, VIII, IX, X, XI, and XII were within normal limits.

She underwent knee surgery and was given cryoprecipitate postoperatively as prophylaxis against bleeding. Her fibrinogen level (activity) went up to 217 mg/dL. She experienced no perioperative bleeding.

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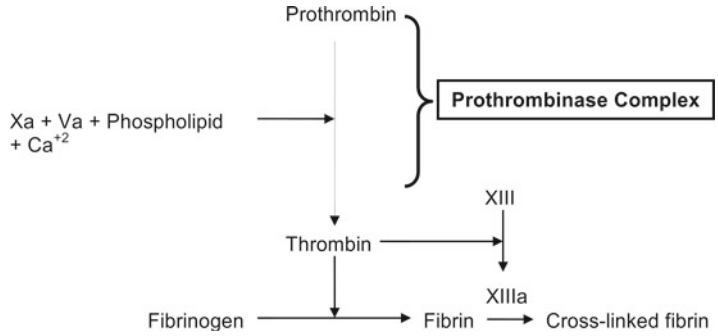

Fig. 5.1 Coagulation cascade—common pathway

Table 5.1 Causes of a combined prolongation of the PT and aPTT

Inherited	Acquired
Afibrinogenemia	Vitamin K deficiency
Dysfibrinogenemia	Liver disease (decreased synthesis or dysfibrinogenemia)
Prothrombin deficiency	Acquired inhibitors: Prothrombin, factor V, factor X
Factor X deficiency	Amyloidosis
Factor V deficiency	Lupus anticoagulant
Combined FV and FX deficiencies	

Fibrinogen is a large glycoprotein (340 kDa) encoded by three genes located in close proximity to each other on chromosome 4. It is synthesized in the liver, and its normal plasma concentration is approximately 250 mg/dL (Marder et al. 2013). A level of 50–100 mg/dl is considered adequate to support hemostasis. Compared to most coagulation factors, it has a long half-life of 3–5 days, so that replacement with cryoprecipitate in a patient with congenital hypofibrinogenemia can be relatively infrequent. Fibrinogen is a complex molecule, consisting of three different paired polypeptide chains expressed as $A\alpha_2$, $B\beta_2$, and γ_2 that are linked together by disulfide bonds (Mosesson et al. 2001). During the process of fibrin formation and stabilization these fibrinopeptides are cleaved by thrombin, forming fibrin which then polymerizes. The fibrin polymers must be cross-linked through the action of factor XIII (activated by thrombin) resulting in a stable clot at the site of a vascular injury. Remodeling of the clot then occurs through activation of tissue-bound plasminogen by tissue plasminogen activator, generation of plasmin, and cleavage of the stabilized fibrin meshwork within the clot. Besides its function in the coagulation cascade, fibrinogen facilitates platelet aggregation via binding to the platelet glycoprotein IIb/IIIa ($\alpha_{IIb}\beta_3$) receptor and von Willebrand factor (Marder et al. 2013).

Conversion of fibrinogen to fibrin by thrombin is the most distal step in the coagulation cascade. In the laboratory this step is measured in isolation by either the thrombin or the reptilase times. The thrombin time is sensitive to the presence of heparin, whereas heparin has no effect on the reptilase time in which the reagent used to initiate the reaction is a snake venom enzyme, reptilase. This allows assessment of fibrinogen function even in the presence of heparin. Both the thrombin time and reptilase times will be elevated in either hypofibrinogenemia or dysfibrinogenemia, but the thrombin time is more sensitive for detecting dysfibrinogenemia (Hayes, 2002). Although an absence of fibrinogen will affect both coagulation times (PT and aPTT), dysfibrinogemia appears to have a preferential effect on the prothrombin time (Hayes 2002).

Two major methods are used to quantify the level of functional fibrinogen, the PT-derived method and the Clauss assay. Both of these are clot-based methods, and the detection limit is variable (20–60 mg/dL). In a comparison study, the PT-derived method correlated with the fibrinogen antigen concentration, whereas the Clauss method correlated best with functional coagulation tests such as the reptilase time, thrombin time, and prothrombin time. It was therefore

recommended that the Clauss assay be used in the management of hypofibrinogenemic patients who are bleeding (Miesbach et al. 2010).

While quantitative and qualitative abnormalities of fibrinogen are the most common heritable causes of a combined PT and aPTT elevation, they themselves are rare. Afibrinogenemia occurs with a frequency of 0.0001 % and hypo- and dysfibrinogenemia at a somewhat higher frequency (Acharya et al. 2004). The latter are difficult to quantify because many patients, especially with dysfibrinogenemia, are asymptomatic.

Afibrinogenemia, defined by the complete absence of measurable fibrinogen activity and antigen, is inherited in an autosomal recessive fashion with variable penetrance (Asselta et al. 2006). The majority of the causal mutations have been discovered in the fibrinogen Aα, or *FGA* gene, but others have been found in the Bβ and -γ chains resulting in either a complete loss of expression or production of abnormal proteins that are retained within the cell. The gene defects include deletions, frameshift, nonsense, or splicing mutations (de Moerloose and Neerman-Arbez 2009). Homozygous mutations usually lead to the most severe hemorrhagic tendency. However, correlations between phenotype and genotype are not always predictable. Hypofibrinogenemic patients (fibrinogen activity levels below 150 mg/dL with a proportionate decrease in fibrinogen antigen) are usually heterozygous for the same mutations found in afibrinogenemia.

Dysfibrinogenemia results from production of a qualitatively dysfunctional fibrinogen molecule. It is transmitted with an autosomal dominant mode of inheritance. Dysfibrinogenemia can also be acquired in patients with liver disease. In the inherited form, causative mutations, mostly in *FGA*, cause defective cellular release or abnormal fibrin polymerization and cross-linking. In typical cases the fibrinogen functional activity is low, but the immunological measurement reveals a normal amount of fibrinogen antigen, usually twice that of the activity level. Other laboratory findings include correction of the abnormal PT and aPTT in mixing studies, elevations in both the thrombin and reptilase times, and normal levels of coagulation factors in the extrinsic and intrinsic pathway.

Clinical Manifestations

The bleeding symptoms in afibrinogenemia are more severe than in either hypofibrinogenemia or dysfibrinogenemia but generally less severe than in hemophilia. The types of bleeding also differ in that there is a lower incidence of joint and muscle bleeds and a higher incidence of mucosal bleeding than in hemophiliacs. The most common kinds of bleeds in afibrinogenemic patients include umbilical cord bleeding (85 %), muscle hematoma (72 %), mucosal bleeding (including epistaxis, oral cavity, and menorrhagia) in 72 %, hemarthrosis (54 %), and CNS hemorrhage (10 %) (Lak et al. 1999). Posttraumatic hematomas are also common in these patients. This distribution of bleeding sites is similar to that of more recent series of 90 patients in Iran (Lak et al. 2010). Afibrinogenemia is also associated with thrombotic events and an increased incidence of spontaneous abortion, particularly in the first trimester.

Patients with congenital dysfibrinogenemia have a milder disease. Approximately 55 % may be asymptomatic and go undiscovered until screening coagulation testing is done in preparation for surgery. About 25 % have a bleeding tendency, and 20 % have thromboembolic complications (de Moerloose et al. 2010).

Management

Management of the patient with congenital afibrinogenemia consists of prophylactic replacement of fibrinogen. This typically consisted of regularly scheduled infusions of cryoprecipitate for both prophylaxis and at the time of a bleed or a procedure. In Europe and now the USA, a plasma-derived fibrinogen concentrate is approved for use for acute bleeding episodes in afibrinogenemic patients (RiaSTAP®). The fibrinogen concentrates are favored since dosing is more defined, extraneous coagulation proteins (factor VIII, von Willebrand factor, factor XIII) are avoided, and they are virally inactivated. However, thrombosis has been associated with the administration of such concentrates (Bornikova et al. 2011).

In general, there is a good correlation between the functional fibrinogen level and the occurrence of bleeding, with the accepted hemostatic level of 50 % (Castaman 2008). However, the optimal dosage and target level of fibrinogen for prophylaxis are unknown. The severity and frequency of bleeding must be taken into account when deciding on the need for secondary prophylactic replacement therapy. A prior CNS bleed or frequent spontaneous bleeding are the two most common indications for prophylaxis. Fibrinogen concentrate or cryoprecipitate are given with variable frequency, from every week to every 2 weeks to monthly (Peyvandi et al. 2006). In a large review of case reports of treated patients, a fibrinogen level of 50–100 mg/dL was most commonly used as a goal of replacement for nonsurgical patients and 100–200 mg/dL in surgical cases (Bornikova et al. 2011). For epistaxis or dental extractions, antifibrinolytics such as epsilon aminocaproic acid (EACA) or tranexamic acid can be used as adjunctive therapy.

There are several reports of success with fibrinogen replacement to prevent miscarriage in afibrinogenemic women (Frenkel et al. 2004). Again, the schedule and target level of fibrinogen are not well defined, although a target trough level was typically above 60 mg/dL (Bornikova et al. 2011).

Prothrombin, Factor V, and Factor X Deficiencies

Clinical Vignette 2

RT is a 64-year-old female admitted for left lower quadrant pain, nausea, and vomiting. She gave the history of multiple bleeding episodes since she was a child, including umbilical cord hemorrhage and severe bleeding when she was losing her baby teeth. She was hospitalized on several occasions and required transfusion. As an adult, she had severe bleeding after dental extraction treated with EACA and topical thrombin. She has had multiple episodes of hemarthrosis in her knees, wrists, ankles, and elbows. She was managed only with bed rest. She had severe menorrhagia from the time of menarche and had to be transfused with PRBCs and plasma in the past. Depoprovera was given to suppress her periods. Her brother had had multiple episodes of hemarthrosis and also bleeding with the loss of teeth. They were both given vitamin K as children for bleeding episodes. She is one of 12 children, but none of her siblings had a bleeding diathesis.

There was no history of cardiac, pulmonary, renal, hepatic, and thyroid disease; diabetes; or hypertension. She is G0, P0.

Laboratory testing: PT >76.9 (8.4–13 s)
1:1 mix with normal plasma, 11.9 s
aPTT 76.3 (23–32.4 s)
Fibrinogen clot 784 (200–400 mg/dL)
Thrombin time 18.3 (<18.6 s)
Factor X <1 % (73–163 %)
Factor II 70 % (71–138 %)

She received two units of FFP in preparation for colonoscopy. She did not require surgery.

Prothrombin, factor V, and factor X are all positioned in the common distal pathway of coagulation, and their deficiencies lead to combined elevations in both the PT and aPTT (see Fig. 5.1). Because of their rarity these deficiencies have been considered as part of a group of "rare bleeding disorders" (RBD) or "rare inherited coagulation disorders" (RICD). All have an autosomal recessive inheritance pattern and occur with a prevalence of <1:1,000,000 in homozygous forms, which are frequently associated with consanguinity. The gene mutations or deletions that have been identified usually lead to quantitatively defective synthesis of each corresponding factor (i.e., a type 1 deficiency). In combined factor VIII and V deficiency, however, the defect is neither of these gene sequences, but rather in a gene responsible for intracellular

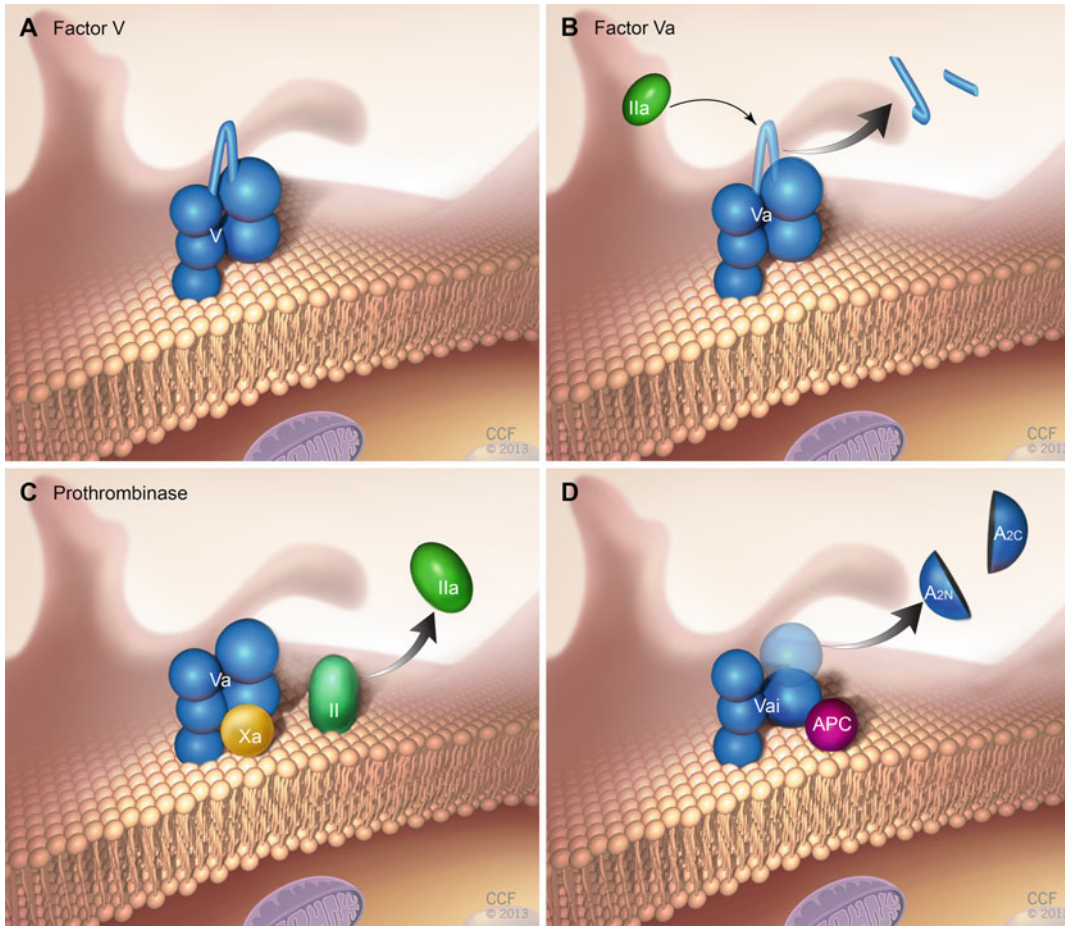

Fig. 5.2 Activation of factor V and formation of the prothrombinase complex. (**a**) Inactive factor V binds to the phospholipid surface. (**b**) Factor V is activated to factor Va by thrombin (IIa). (**c**) Factor Va binds to factor Xa forming the prothrombinase complex which cleaves prothrombin (II) to thrombin (IIa). (**d**) Activated protein C (APC) cleaves at three sites of the factor Va molecule, rendering it inactive (Vai). The resulting product (factor Vai) is unable to generate thrombin (adapted from Mann and Kalafatis 2003)

transport and secretion of the factors from the cell. These genes are MCFD1 or LMAN1, and the end result of the mutation is a decrease in FV and FVIII activities to approximately 5–30 % accompanied by a moderate bleeding disorder. Inherited combined deficiencies of all vitamin K-dependent (VKD) factors have also been reported. In the few patients that have been described, the molecular defects have been found in the γ-glutamyl carboxylase gene necessary for the carboxylation step in the posttranslational modification of all VKD factors and in the vitamin K epoxide reductase gene, responsible for regeneration of reduced vitamin K (Brenner 2000). Interestingly, in animal models the defect caused by defective carboxylation can be bypassed by supplemental vitamin K.

Prothrombin and factor X are synthesized in the liver and circulate as precursors to a serine protease. Factor V is also thought to be synthesized in the liver, but there is evidence of synthesis in megakaryocytes as well. Approximately 20 % of plasma factor V is stored in platelet α-granules (Tracy et al. 1982). Their activities intersect within the prothrombinase complex where factor Xa cleaves prothrombin to thrombin in the presence of factor Va, calcium, and phospholipid (Fig. 5.2). Factor Va increases the activity of factor Xa by about 5 orders of magnitude (Mann and Kalafatis 2003).

Clinical Manifestations

While these factors play essential roles within the coagulation cascade, their deficiency, even when severe, is not always manifested by a bleeding diathesis. The bleeding complications are quite variable. As with afibrinogenemia, the distribution of bleeding sites varies from that of hemophilia with a lower incidence of joint, muscle, and CNS bleeding (Mannucci et al. 2004). Menorrhagia is common in women with these deficiencies as are other types of mucosal bleeding (oral cavity, epistaxis).

Factor V deficiency, initially known as "parahemophilia," mainly presents with platelet-type bleeding such as ecchymoses, epistaxis, menorrhagia, and bleeding after surgery. This is perhaps due to the involvement of platelets as a source of factor V for the prothrombinase complex. The complete absence of factor V in mice causes severe hemorrhage, but in humans with levels of plasma factor V activity of <2 % there may be no bleeding tendency. In a registry of RBD in North America, a cohort of 18 patients with severe deficiency (median factor V level of <0.01 U/ml) had spontaneous hemorrhage including hemarthrosis and intramuscular and intracranial bleeds. In those who were heterozygous (median factor V level of 0.35 U/ml), there was a lower incidence of spontaneous skin, mucous membrane, musculoskeletal, and genitourinary bleeding (Acharya et al. 2004).

Patients who are homozygous for factor X deficiency have a median factor X level of <0.01 U/ml and have a similar incidence of cutaneous and mucous membrane bleeding (45 %) and musculoskeletal bleeding (27 %) but a slightly higher rate of CNS bleeding (15 %) compared to severely deficient factor V patients (Acharya et al. 2004). Most patients have missense mutations (Mannucci et al. 2004; Herrmann et al. 2006). This distribution of bleeding sites is based on a small number of patients. Herrmann et al. described a larger cohort correlating the clinical and laboratory phenotype with mutational analysis of the factor X gene (Herrmann et al. 2006). Spontaneous bleeding was seen in both homozygous and heterozygous patients, but severe hemorrhage (CNS, GI, hemarthrosis) occurred only in patients with factor X levels of <2 %. For heterozygous patients, factor X coagulation activity in the plasma correlates imperfectly with clinical bleeding. For example, even though the mean factor X level of the heterozygous patients was 50.7 %, 13 % of them were symptomatic with spontaneous epistaxis, menorrhagia, and bruising. However, none had the severe forms of bleeding seen in homozygous patients. This is somewhat higher than the previously reported incidence of bleeding in heterozygous patients (Uprichard and Perry 2002).

Deficiency of prothrombin is the rarest of the congenital bleeding disorders. Severe deficiency (median activity of 0.03 U/ml) resulted in either spontaneous or trauma-induced bleeding with a distribution of sites similar to that of FV deficiency: skin, mucous membrane, musculoskeletal, GI, GU, and CNS. Those with moderate deficiency (less than 0.25 U/ml) had bleeding episodes which were in the skin and mucous membranes (Acharya et al. 2004). The PT and aPTT are sensitive enough to detect factor V, factors V + VIII, and factor X deficiencies but may be normal in prothrombin deficiency.

Management

Specific replacement of the deficient factor in the rare bleeding disorders is the ideal treatment for bleeding episodes as it is in hemophilia A and B. Unfortunately, neither concentrates nor recombinant proteins are available for these rare deficiencies. The most specific replacement at this time for the VKD factors is with prothrombin complex concentrates (PCCs), containing either 3 (II, IX, and X) or 4 (II, VII, IX, and X) coagulation factors. PCCs were originally licensed for treatment of hemophilia B but are now used for warfarin reversal and as bypass agents in the treatment of factor VIII inhibitors, either acquired or in hemophiliacs. The 4-factor PCC available in North America, FEIBA®, is in an activated form, but all PCCs are thrombogenic, especially after high cumulative doses. Monitoring of the nonessential factors is recommended to avoid levels of >150 %

Table 5.2 Hemostatic levels and half-lives of coagulation factors

Deficient factor	Hemostatic level	Plasma half-life
Fibrinogen	50–100 mg/dL	2–4 days
Prothrombin	20–30 %	3–4 days
Factor V	15–20 %	36 h
Factor X	15–20 %	40–60 h

Modified from Mannucci et al. (2004)

(Mannucci et al. 2004). They have the advantage over plasma of delivering a more precise amount of either prothrombin or factor X (although labeled in units of factor IX, amounts of other factors are available from the manufacturer). Another advantage is that less volume is required compared to plasma.

Treatment dosage and schedule depend upon the type of bleeding or surgical procedure as well as the target level and projected half-life of the factor being replaced. Prothrombin and factor X deficiencies can be treated with PCCs or FFP (Mathias et al. 2010). Prothrombin has a long half-life (see Table 5.2), so dosing is less frequent. The recommended dose of PCC for major surgery is 20–30 U/kg and for FFP, 15–20 ml/kg for both prothrombin and factor X deficiencies (Mannucci et al. 2004). Factor V deficiency can only be replaced with FFP since no concentrate is available. The recommended dose is 15–20 ml/kg to raise the factor V level to >20 % (Mannucci et al. 2004). The half-life of factor V, 36 h, allows once-a-day dosing with FFP. Recombinant factor VIIa (rFVIIa) has also been used in factor V-deficient patients.

Acquired Defects Prolonging the PT and APTT

Liver Disease

Since the liver is the sole site of synthesis for all coagulation factors with the exception of factor VIII and VWF, it stands to reason that hepatic dysfunction will lead to reduced factor synthesis and prolongation of the screening coagulation tests. Despite the addition of thrombocytopenia and numerous other hemostatic abnormalities, this does not necessarily imply an increased bleeding risk. Conversely, protection from thrombosis cannot be assured because of the concomitant reductions in protein C, protein S, and antithrombin production as well as other prothrombotic changes that occur in liver failure. Indeed thrombosis is not uncommon in advanced hepatic disease, ranging in incidence from 0.5 to 1.9 % and up to 6.3 % in hospitalized patients (Dabbagh et al. 2010; Rodriguz-Castro et al. 2012).

In liver disease, either acute hepatitis or more chronic conditions, the PT is more sensitive to the effects of the coagulopathy perhaps due to the greater proportionate decrease in factor VII compared to the other VKD factors, II and X, which, in turn, are decreased to a greater degree than factor IX. This is due to alterations in synthesis as well as impaired utilization of vitamin K resulting in decreased carboxylation of factor II (Corrigan et al. 1982; Blanchard et al. 1981). Abnormalities of fibrinogen, either hypofibrinogenemia as a result of impaired synthesis or dysfibrinogenemia, are seen mainly in chronic hepatitis and cirrhosis. This form of acquired dysfibrinogenemia has been related to an abnormal sialic acid content (Marder et al. 2013). The fibrinogen level is less than normal but usually greater than 100 mg/dL. Thrombin and reptilase times will be prolonged.

Vitamin K Deficiency

Vitamin K is a critical cofactor in the synthesis of the phospholipid-dependent coagulation factors II, VII, IX, and X and the endogenous inhibitors, protein Z and protein C and its cofactor protein S. It allows for the posttranslational modification of glutamic acid residues to γ-carboxylated glutamyl or Gla residues at the N-terminal regions of these molecules through the action of γ-glutamyl carboxylase. This enables the VKD factors to bind calcium, a necessary step in their activation. Vitamin K is also important in the modification and activation of other Gla proteins important in bone metabolism (osteocalcin), inhibition of arterial calcification (matrix Gla protein or MGP), and cellular growth

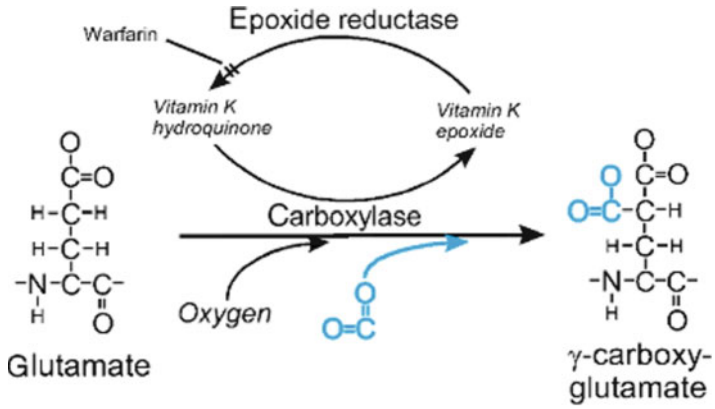

Fig. 5.3 Vitamin K acts as a cofactor in the γ-carboxylation of glutamic acid residues within coagulation factors and other Gla-containing proteins. *Source*: Lichtman MA, Beutler E, Kipps TJ, Seligsohn U, Kaushansky K, Prchal JT: Williams Hematology, seventh Edition: http://www.accessmedicine.com. Copyright © The McGraw-Hill Companies, Inc. All Rights Reserved

regulation (growth-arrest-sequence protein 6 or Gas6). Vitamin K deficiency has been implicated in postmenopausal bone loss and in atherosclerosis (Berkner and Runge 2004). In total 17 Gla proteins have been discovered at this time (Berkner and Runge 2004).

The reduced form of vitamin K (vitamin K hydroquinone or KH2) serves as the cofactor for γ-glutamyl carboxylase and is recycled to vitamin K epoxide. Vitamin K epoxide is then converted back to KH2 by vitamin K oxidoreductase or VKOR (see Fig. 5.3).

Carboxylation of the glutamic acid residues in the VKD coagulation factors is necessary for binding to anionic phospholipids on whose surface the central reactions of the coagulation cascade take place, i.e., formation of the tenase and prothrombinase complexes. Without vitamin K, those factors are still present but nonfunctional and known as Proteins Induced by Vitamin K Absence, or PIVKA.

Rare combined deficiencies of the VKD factors occur with hereditary deficiency of either γ-glutamyl carboxylase or VKOR. These patients have very low levels of factors II, VII, IX, and X. Severely affected individuals have life-threatening intracranial or umbilical cord bleeding in infancy or spontaneous joint and soft tissue bleeding in childhood if factor levels are below 5 U/dL (Brenner et al. 2009). Skeletal changes and mental retardation can occur. Both the PT and PTT are prolonged without evidence of liver disease or nutritional insufficiency.

Acquired coagulopathy due to vitamin K deficiency occurs in various settings, most commonly in the hospitalized, severely ill patient who has poor nutritional intake, malabsorption, inflammatory bowel disease after extensive intestinal resection, biliary obstruction or undergoing broad-spectrum antibiotic therapy. Infants are most susceptible due to poor placental transport of vitamin K and the low concentration of the vitamin in breast milk (Lippi and Franchini 2011). However, production of vitamin K by intestinal bacteria, once thought to be an important source of the vitamin, is now felt to be insufficient for the normal requirement and possibly not available for use in hemostatic and other reactions. Moreover the normal intestinal flora does not produce enough vitamin K to replace that lost when there is a decrease in delivery of bile salts into the intestinal tract (Berkner and Runge 2004; Lippi and Franchini 2011).

Compared to the non-hemostatic functions supported by vitamin K, the amount necessary for adequate hemostasis is relatively small, and some would argue that the minimum daily requirement (100 μg per day) should be increased

(Berkner and Runge 2004). This is especially true in hemodialysis patients who have high levels of non-carboxylated MGP on 140 μg vitamin K, a situation which may put them at risk of increased arterial calcification and vascular disease (Cranenburg et al. 2012).

Assessment of vitamin K status in hospitalized patients is usually by means of global coagulation tests, the PT and aPTT. However, these are very insensitive to vitamin K deficiency since the prothrombin level needs to be only 40–50 % of normal to result in a normal PT. Both increased levels of the non-carboxylated, nonfunctional form of prothrombin (PIVKA-II) and phylloquinone can be measured and used as indicators of vitamin K deficiency (Shearer 2009). In liver disease this becomes more complex since it is not possible to separate, by measurement of the PT and aPTT, defective synthesis of coagulation factors due to hepatic failure from the reduced VKD factor activity due to vitamin K deficiency. Phylloquinone levels may be useful in this circumstance but are not readily available. An easier approach is to first do mixing studies on the PT and aPTT to rule out inhibitory activity. Once an inhibitor is ruled out, measurements of representative VKD factors can be compared to factor V, a non-VKD factor synthesized in the liver. If the level of a VKD factor (II, VII, or X) is low but factor V is normal, the diagnosis is vitamin K deficiency. If both are low, deficient factor synthesis from liver failure is more likely.

Replacement of vitamin K can be done in various ways. Oral replacement is adequate provided the patient has a functional GI (and biliary) tract. In hepatic parenchymal disease, oral vitamin K was less effective than IV when assessed by phylloquinone and PIVKA-II levels (Pereira et al. 2005). Similarly, postoperative or severely ill patients in the ICU have GI absorptive defects, so vitamin K must be given parentally. Subcutaneous or intravenous dosing, 1–10 mg, is sufficient. While anaphylactic reactions have been reported after parenteral administration, these are related to the stabilizer or the emulsifier in the vitamin K preparation rather than vitamin K itself (Shearer 2009).

Lupus Anticoagulants

Although the aPTT is a relatively insensitive test for the lupus anticoagulant (LA), it is the typical laboratory presentation of this phenomenon. Unless the patient is on warfarin, the PT is usually normal. However, there are two circumstances when both the PT and aPTT will be elevated, although not necessarily proportional to each other in the presence of a lupus anticoagulant. This results from a deficiency in prothrombin, either through antibody-mediated increased prothrombin clearance or specific neutralizing antiprothrombin antibodies (Bajaj et al. 1983). This is known as lupus anticoagulant-hypoprothrombinemia syndrome (LAHS) and is a rare variant of the LA which presents with bleeding instead of clotting manifestations due to low prothrombin levels. According to a recent review, 74 cases of LAHS have been reported, with 89 % presenting with bleeding manifestations, including severe hemorrhage in the brain, GI tract, and soft tissue (Mazodier et al. 2012). Most cases are associated with autoimmune disease, particularly SLE. In children, infection is the most common cause with usual resolution of LAHS after the infection clears.

In the laboratory, the aPTT is variably elevated; the PT is increased but proportionately less. The prothrombin activity and antigen levels are severely decreased, consistent with increased clearance of antibody–antigen complexes (Mazodier et al. 2012). Antiprothrombin antibodies are common in LA-positive patients and may prolong the PT while not causing either a decrease in plasma prothrombin activity or bleeding symptoms. These antibodies were found to react in a polyspecific fashion with both negatively charged phospholipids and prothrombin (Fleck et al. 1988). It is still controversial whether these antiphospholipid/prothrombin antibodies play a significant role in the clinical manifestations of antiphospholipid syndrome (Hoxha et al. 2012; Nojima et al. 2001; Marozio et al. 2011; Pengo et al. 2010).

Unlike most cases of a typical lupus anticoagulant, significant steroid responsiveness has been reported in LAHS, with normalization of the PT and prothrombin level occurring in approximately 60 %. Other immunosuppressive therapy with azathioprine and cyclophosphamide is used in patients who have LAHS in association with SLE. There are too few cases reported to assess response to other immune-modulating treatment such as IVIG and rituximab (Mazodier et al. 2012).

Acquired Factor V Inhibitor

Acquired inhibitors to coagulation factors in the common distal pathway that lead to both PT and PTT prolongation are rare events, occurring in even lower frequency than factor VIII autoantibodies. Perhaps the best known is the inhibitor to factor V that can develop after exposure to bovine thrombin. Topical thrombins have been in use for decades, particularly in vascular, cardiac, and neurosurgical procedures. Cross-reactivity of anti-bovine thrombin antibodies with human thrombin is known to occur, causing a prolonged thrombin time, PT, and PTT but usually no significant bleeding. Serum IgG bound to thrombin and prothrombin has been demonstrated in these cases (Stricker et al. 1988). In early reports of acquired factor V inhibitors, most patients had had a major surgical procedure just prior to detection of the inhibitor, and transfusion or antibiotics were implicated. It is unknown how many of them were exposed intraoperatively to topical bovine thrombin (Feinstein 1978). A bovine thrombin-induced factor V inhibitor was first described in 1990 in a cardiac surgery patient who had severe bleeding 12 days after his surgery. The factor V activity was 1 % of normal, and mixing studies of his elevated PT, PTT, and thrombin time showed no correction with normal plasma. While his serum IgG reacted with bovine and not human thrombin, there was reactivity with both bovine and human factor V, indicating that the anti-bovine factor V antibody cross-reacts with human factor V (Zehnder and Leung 1990).

Multiple additional cases of thrombin-induced factor V inhibitors have since been reported with variable bleeding manifestations from severe fatal hemorrhage to no excessive bleeding (Rapaport et al. 1992; Spero 1993; Streiff and Ness 2002). Anti-factor V antibodies resolve over time, but if severe bleeding occurs aggressive management is necessary. Treatment with steroid, IVIG, cyclosporine, cyclophosphamide, EACA, and plasmapheresis has been attempted with variable degrees of success (Zehnder and Leung 1990; Streiff and Ness 2002). The development and FDA approval of recombinant human topical thrombin should obviate this problem. It appears to be equivalent in efficacy to bovine thrombin and is less immunogenic (Chapman et al. 2007; Singla et al. 2009).

In addition to inhibitors seen after the use of bovine thrombin, acquired factor V inhibitors have been observed after other drug exposures such as amiodarone (Shreenivas et al. 2012), in patients with malignancy (Nesheim et al. 1986), after liver transplantation (Guglielmone et al. 2011), and as spontaneous or postoperative events (Bobba et al. 2011). However, it is difficult to assign a cause of the inhibitor formation as infection, antibiotic exposure, and other autoimmune conditions are often found in the background of reported cases of factor V and other acquired inhibitors (Franchini and Lippi 2011).

These inhibitors can result in serious bleeding from the GI and GU tracts and other mucosal surfaces (Lipshitz et al. 2012), but in recent reviews, 20–30 % are discovered incidentally in the absence of hemorrhage (Franchini and Lippi 2011; Ang et al. 2009). The frequency of bleeding in spontaneous factor V inhibitor patients, however, appears to be about twice that seen in bovine thrombin-induced inhibitors (Streiff and Ness 2002).

The diagnosis of a spontaneous factor V inhibitor in the laboratory is similar to bovine thrombin-induced ones—the PT and PTT will be prolonged without correction in mixing studies,

and factor V levels will be low. A Bethesda assay on factor V will quantify the inhibitor and will inactivate factor V in minutes, although some inhibitors are time dependent. Two hours of incubation are recommended (Ang et al. 2009). In two reviews of acquired factor V inhibitors, the level of inhibitor titer was not predictive of bleeding. Patients with bleeding manifestations, however, had lower factor V levels—a median of 1 % in bleeding patients and 3 % in non-bleeders—and higher PT and aPTTs (Franchini and Lippi 2011; Ang et al. 2009).

As with other acquired factor inhibitors, treatment is designed in two phases—control of the bleeding and eradication of the inhibitor. Fresh frozen plasma, PCCs, rFVIIa, and platelet transfusions have been used with variable degrees of success. Plasma is not expected to be effective due to rapid factor V inactivation by the patient's antibody. This may not be the case with platelet transfusion in which the factor V contained within platelets may be protected from the inhibitor. In some series, a fairly high rate of response to platelet concentrates is reported (Ang et al. 2009; Chediak et al. 1980), but this has not been a uniform experience (Bobba et al. 2011).

Suppression of the inhibitor can usually be accomplished by various immune-modulating agents. Corticosteroids are the most common initial therapy and result in a high response rate. As with acquired factor VIII inhibitors, rituximab has also been used successfully (Ang et al. 2009; Lebrun et al. 2008; Lian et al. 2004). Other immunosuppressant drugs such as azathioprine, cyclophosphamide, and vincristine should be used only if less toxic agents are ineffective. Factor V inhibitors have been observed to resolve spontaneously in 20–56 % of patients, particularly in those in whom an identifiable cause (e.g., antibiotic therapy) can be removed. The median time to resolution is 6–8 weeks. Although a mortality rate from bleeding complications of 12 % has been reported, the overall prognosis of a factor V inhibitor is largely dependent on that of the underlying disease (Franchini and Lippi 2011).

Acquired Factor X Deficiency and Inhibitors

Clinical Vignette 3

DH is a 37-year male who was well except for a history of hypertension. He sustained abdominal trauma playing with his daughter and developed intraperitoneal hemorrhage requiring exploratory laparotomy, splenectomy, and segmental liver resection. Subconjunctival hemorrhage was noted. Three months later he presented with spontaneous flank pain and hematemesis and had a large perinephric hematoma associated with acute renal failure. Biopsies of both the duodenum and stomach contained deposits of acellular amorphous eosinophilic material, congophilic on Congo red stain with an apple-green birefringence under polarized light. An immunohistochemical stain for P-component was reactive within the deposits confirming that the material was amyloid.

Further pathological review of the spleen also revealed Congo red-positive material confirmed to be amyloid. Immunohistochemical stains for kappa and lambda light chains showed preferential staining of the amyloid material for lambda. A bone marrow biopsy showed plasmacytosis with lambda light chain excess and aberrant cyclin D1 expression, consistent with involvement by a plasma cell neoplasm.

Laboratory testing:
PT 15.7 (8.4–13 s)
1:1 mix with normal plasma, 11.5 s
aPTT 32.7 (23–32.4 s)
Fibrinogen clot 475 (200–400 mg/dL)
Factor X 28 % (73–163 %)
Factor II 98 % (71–138 %)
Factor V 89 % (50–150 %)
Factor VII 72 % (50–150 %)

Treatment was initiated with bortezomib, dexamethasone, and EACA. His prothrombin time corrected to normal.

(continued)

Ultimately the kappa-lambda serum levels and ratio normalized, and a repeat bone marrow biopsy showed no evidence of myeloma. However an echocardiogram demonstrated severe LVH, stage 2 diastolic dysfunction, increased RV wall thickness, and an estimated RVSP of 43 mmHg, consistent with mild pulmonary hypertension; the overall appearance was consistent with cardiac amyloidosis. He underwent high-dose melphalan therapy followed by autologous peripheral stem cell transplantation. He has been in hematologic remission over the subsequent 2 years without further episodes of bleeding. His PT and aPTT are normal, and the factor X level has improved to 62 %.

Aside from liver disease and vitamin K deficiency, acquired factor X deficiency is usually due to systemic AL amyloidosis. In two large series, low factor X levels occurred in 8.7–14 % of amyloidosis patients (Choufani et al. 2001; Mumford et al. 2000). A significant reduction in factor X activity but not necessarily factor X antigen is found, without detectable inhibitory activity. The half-life of factor X after infusion of plasma is extremely low due to increased clearance of factor X from the circulation via binding to amyloid fibrils (Furie et al. 1977). The amount of exposed amyloid in the circulation and the particular binding characteristics of an individual patient's type of amyloid determine how much factor X is removed and whether factor X deficiency and bleeding occur (Furie et al. 1981). Other data, showing discordance between low factor X coagulant activity and normal factor X antigen levels, suggest some other mechanism resulting in defective factor X function (Mumford et al. 2000).

Approximately a third of all patients with AL amyloidosis and 56 % of those with factor X deficiency will have bleeding symptoms. Subcutaneous and gastrointestinal bleeding are the most common sites, and splenic hemorrhage has been described. The severity of bleeding (other than in the skin) appears to parallel the level of factor X activity (Choufani et al. 2001; Mumford et al. 2000). Of the global coagulation screening tests, a prolonged thrombin time (32 %) and PT (24 %) were most commonly found, with a prolonged PTT seen in 14 % of amyloid patients; the thrombin time does not correlate with bleeding but is associated with proteinuria and hypoalbuminemia (Mumford et al. 2000).

Besides factor X deficiency, other coagulation defects have been described in amyloidosis. Included is factor V deficiency, probably due to increased factor V clearance through binding to amyloid as described with factor X. The deficiency in factor V can cause significant bleeding (Emori et al. 2002). Amyloid binds to factor IX and prothrombin (to a lesser extent than factor X), causing subnormal plasma levels (Furie et al. 1981). Increased vascular fragility, abnormal fibrin polymerization, and dysfibrinogenemia have been described (Mumford et al. 2000).

Treatment of amyloid-associated bleeding which is due to factor X deficiency is difficult. Plasma has little, if any, effect due to factor X's rapid binding to the blood vessel-contained amyloid. Activated PCCs, rFVIIa, and plasma exchange have been used to treat bleeding and in preoperative management of amyloidosis patients (Thompson et al. 2010; Boggio and Green 2001; Ma et al. 2006). In a retrospective review of surgical outcomes in patients with amyloidosis, there was a 10 % incidence of bleeding complications, more commonly with placement of a central venous catheter. Surprisingly, the incidence of bleeding did not correlate with factor X levels, implying the presence of other hemostatic defects which modify bleeding risk. In nine patients in whom rFVIIa was used, four (44.4 %) had either bleeding or thrombotic complications, raising caution about the use of rFVIIa in this patient population (Thompson et al. 2010). Splenectomy has resulted in improved factor X levels and resolution of bleeding, perhaps due to removal of the large

splenic reservoir of amyloid protein resulting in a decrease in factor X clearance (Ma et al. 2006; Rosenstein et al. 1983). However, this is a high-risk procedure in amyloidosis and should only be considered in a patient with life-threatening bleeding. Primary treatment of AL amyloidosis with chemotherapy and autologous transplantation can lead to correction of factor X deficiency if hematologic remission is achieved (Choufani et al. 2001).

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6 Excessive Bleeding with Normal Prothrombin Time, Partial Thromboplastin Time, and Platelet Count

Senthilkumar Damodaran and Spero R. Cataland

Introduction

The clinical evaluation of a patient with bleeding diathesis begins with a detailed history and physical exam, followed by laboratory testing. A comprehensive history should include the nature, frequency, duration, and severity of bleeding as well as common sites of bleeding such as oral mucosa, gastrointestinal, intramuscular, or intra-articular. In particular, a patient's response to hemostatic challenges in the past, especially surgical procedures and dental extractions, can be quite informative. Also, information regarding interventions performed to stop bleeding should be obtained. Patients with platelet function defects or von Willebrand disease (vWD) may present with bleeding involving the skin and mucous membranes. This usually involves ecchymoses, epistaxis, menorrhagia, gastrointestinal bleeding, as well as excessive bleeding after dental extractions or surgery. Musculoskeletal bleeding such as hemarthrosis is not usually observed. Typically, bleeding with invasive procedures is immediate and seldom life threatening. Severe intracranial or gastrointestinal bleeding is observed more commonly in patients with type-3 vWD. On the other hand, defects or deficiencies in coagulation factors lead to deep tissue bleeding such as intramuscular hematomas and hemarthrosis. They often present with delayed post-procedure bleeding that can be severe.

The platelet count, prothrombin time (PT), and activated partial thromboplastin time (aPTT) are general screening tests used for evaluation of hemostasis. While an abnormality in one of these tests can help delineate an underlying bleeding disorder, often these tests are normal making the diagnosis of a bleeding disorder challenging. A large prospective study in patients with mucocutaneous bleeding identified only about 41 % of patients with vWD, platelet function defects, or clotting factor deficiencies. The remainder had bleeding disorders without an identifiable cause (Quiroga et al. 2007). This usually results in exhaustive testing in an attempt to characterize accurately the bleeding disorder. More common disorders of hemostasis that may present with normal coagulation tests include qualitative platelet disorders, drug-induced platelet dysfunction, factor deficiencies, and abnormal vascular fragility. See Table 6.1 for a listing of such disorders. An algorithm to establish the diagnosis of these disorders is shown (Fig. 6.1).

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Table 6.1 Bleeding disorders with normal coagulation tests

Platelet function disorders
Inherited
Bernard–Soulier syndrome
Glanzmann thrombasthenia
Storage pool disease
Scott syndrome
Acquired
Uremia
Cirrhosis
Paraproteinemias
Drugs
Antiplatelet agents (aspirin, clopidogrel, prasugrel, abciximab, eptifibatide)
NSAIDs
SSRI
Valproic acid
Herbal supplements
Coagulation pathway defects
Factor XIII deficiency
von Willebrand disease
Alpha-2 antiplasmin deficiency
Plasminogen activator inhibitor-1 deficiency
Abnormal vascular fragility
Amyloidosis
Scurvy
Ehlers–Danlos syndrome
Cryoglobulinemia

Qualitative Platelet Disorders

A 50-year-old obese woman who recently underwent bilateral knee replacement surgery was referred for evaluation due to prolonged postoperative bleeding. Her past medical history is significant for hypothyroidism and dyslipidemia. She underwent tonsillectomy as a teen and believes that she had bleeding issues postoperatively. She does report what might have been heavier menstrual cycles in the past. Her physical exam was unremarkable, and laboratory testing demonstrated a mild anemia, but with a normal platelet count and normal PT and aPTT.

Acquired Platelet Disorders

Normal platelet function is contingent on both the quantity and quality of platelets. While quantitative platelet disorders are often easily discerned based on the low platelet counts and evaluation of the peripheral smear, qualitative platelet disorders can often be missed. A detailed history can often provide clues to an underlying disorder. Patients with qualitative platelet disorders tend to bleed immediately after insults and hemostatic challenges and rarely present with serious bleeding unless the underlying defect is severe. While congenital disorders of platelet function are an important cause of bleeding, acquired platelet function disorders are more relevant due to a relatively higher prevalence. Secondary or acquired platelet function disorders can be diagnosed based on their history, duration of symptoms, and associated medical illnesses. Common causes include cirrhosis, uremia, drugs, and hematological malignancies such as myeloma or other paraproteinemias.

While an increased PT, PTT, and thrombocytopenia can be observed in patients with severe hepatic dysfunction, bleeding can be observed even when these tests are normal. This may be due to an acquired platelet dysfunction in patients with cirrhosis. Defects in platelet adhesion along with impaired thromboxane A2 synthesis have been proposed as possible mechanisms (Escolar et al. 1999). Platelet aggregation may be abnormal, and the bleeding time is prolonged in these patients. Uremia also leads to qualitative platelet dysfunction similar to patients with cirrhosis. Usually anemia and thrombocytopenia are also observed in patients with severe renal dysfunction. While the cause for the hemostatic defects is likely multifactorial, defective arachidonic acid pathway and cytoskeletal assembly along with storage pool disorders have been thought to contribute to platelet dysfunction in patients with renal failure (Weigert and Schafer 1998). Ecchymoses, epistaxis, and gastrointestinal and genitourinary bleeding are commonly observed. Symptoms ameliorate with treatment of the underlying disorder, but desmopressin (DDAVP) has been used to help stop or prevent bleeding in

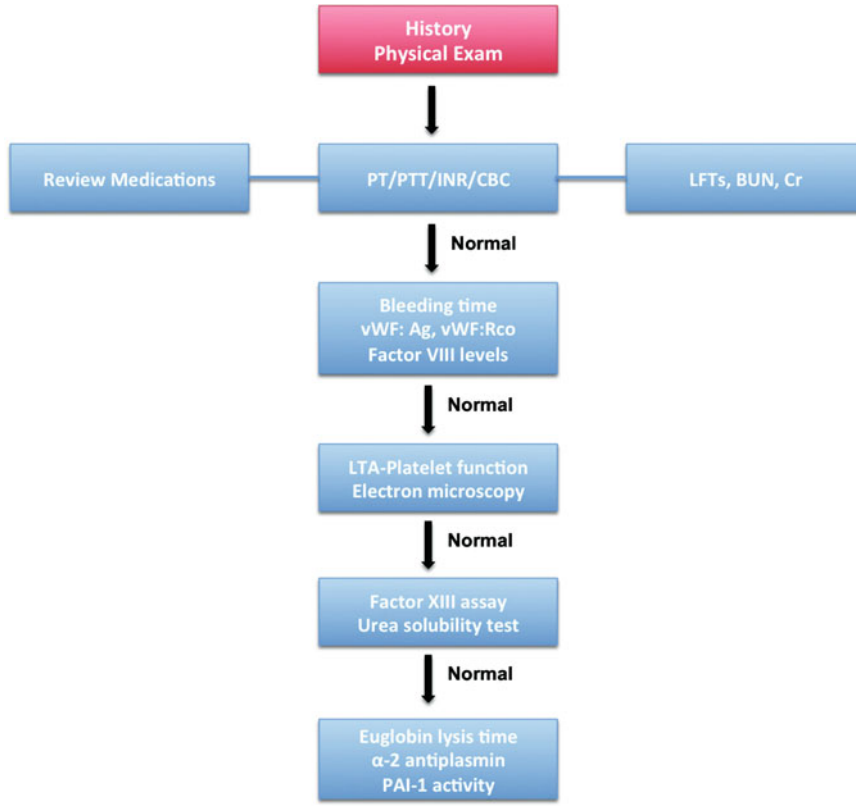

Fig. 6.1 Algorithm to evaluate patients suspected of having an underlying coagulation disorder but with a normal platelet count, PT, and aPTT. *LTA* light transmission platelet aggregometry, *vWF:Ag* von Willebrand antigen, *vWF:Rco* ristocetin cofactor activity

patients with uremia. Cardiopulmonary bypass can also cause significant platelet dysfunction due to hypothermia, complement activation, and a decrease in glycoproteins Ib and IIb/IIIa leading to decreased platelet adhesion to the endothelium and aggregation.

Although the incidence of venous thromboembolism with the use of immune modulators such as lenalidomide has received greater attention, acquired coagulopathies in patients with plasma cell dyscrasias can predispose patients to an increased bleeding risk. The abnormal paraproteins found in patients with multiple myeloma and Waldenström macroglobulinemia can affect all stages of platelet function including adherence, activation, and aggregation. Coating of platelets by antibody, particularly IgM, leads to binding to platelet glycoproteins that can result in platelet dysfunction. In addition to thrombocytopenia, pseudo-thrombocytopenia due to M-protein-induced ex vivo platelet agglutination can be observed (Eby 2009). Acquired vWD, due to absorption of von Willebrand factor (vWF) onto the surface of malignant lymphoid cells aberrantly expressing receptors for vWF and formation of vWF–autoantibody immune complexes, has also been noted (Eby 2009).

Inherited Disorders of Platelet Function

A 53-year-old female was referred for evaluation regarding a possible coagulation disorder to explain her previous procedure-related bleeding complications. Throughout her lifetime she had experi-

(continued)

enced heavy nosebleeds at times, easy bruising, and heavy menstrual cycles. Several years ago she underwent open reduction with internal fixation of a femur fracture and experienced significant bleeding postoperatively that required the administration of packed red blood cells. She subsequently required emergent surgery to relieve a bowel obstruction and was empirically treated with DDAVP preoperatively given anesthesia's concern for a coagulation disorder. She had no postoperative bleeding complications after the bowel surgery in contrast to her previous surgery. Her records indicate that previous coagulation studies including the PT, PTT, ristocetin cofactor activity, and vWF antigen have all been normal.

Bernard–Soulier Syndrome

Inherited qualitative platelet disorders, while not common, are an important cause of bleeding in the face of normal coagulation tests. Bernard–Soulier syndrome (BSS) is a rare autosomal recessive bleeding disorder due to a defect in glycoprotein GP1b-V-IX complex (Lanza 2006). The GPIb–V–IX complex binds vWF allowing platelet adhesion and platelet plug formation following vascular injury. Therefore, a defect in the aforementioned complex can lead to bleeding diathesis with normal coagulation profile and a low to normal platelet count. Typical clinical manifestations include epistaxis, menorrhagia, and gingival and gastrointestinal bleeding. Severe bleeding can occur with trauma and invasive procedures; however, prognosis is usually good with supportive care. The diagnosis of BSS is established based on prolonged bleeding time (PFA) and defective ristocetin-induced platelet agglutination (RIPA) along with a low or absent expression of the GPIb–V–IX complex (Lanza 2006). However, it is often difficult to differentiate BSS from other non-muscle myosin heavy chain (MYH9)-related platelet disorders such as May–Hegglin, Fechtner, Epstein, and Sebastian syndromes that are also associated with macro-thrombocytopenia. The diagnosis of BSS can be corroborated by quantitative analysis of GPIb–V–IX complex (CD42a–d) expression on the platelets or genetic testing (Althaus and Greinacher 2009). Treatment of bleeding associated with trauma or surgery usually involves platelet transfusions. Antiplatelet agents such as aspirin should be avoided. DDAVP and recombination factor VIIa can be used in some patients to ameliorate bleeding. Hematopoietic stem cell transplant is an option in patients with severe life-threatening bleeding (Locatelli et al. 2003). Gene therapy may also be a promising approach for treatment of BSS in future (Kanaji et al. 2012).

Glanzmann Thrombasthenia

Glanzmann thrombasthenia (GT) is an autosomal recessive disorder characterized by a defect in platelet integrin αIIbβ3 (GPIIb/IIIA) resulting in impaired platelet aggregation though it can also be acquired (see below). GT is caused by mutations across the ITGA2B and ITGB3 genes (Nurden et al. 2012). Patients usually present with mucocutaneous bleeding and a normal platelet count without any evidence of platelet clumping. Platelet aggregometry is typically abnormal. GT can occur in combination with defects in leukocyte adhesion. Therefore, newborns with leukocytosis and severe bacterial infections should be evaluated for GT. Patients can present with severe mucocutaneous bleeding and tend to become refractory to platelet transfusions due to development of alloantibodies to GPIIb/IIIA. Recombinant factor VIIa and hematopoietic stem cell transplant have been used in such cases with success (Connor et al. 2008). Acquired GT is rarely observed but may be seen in conditions such as pregnancy, systemic lupus erythematosus, and GPIIb/IIIA antagonist (e.g., abciximab) therapy due to the development of antibodies to the GP IIb/IIIa complex.

Platelet Storage Pool Disease

Storage pool disease is characterized by defects in platelet granules that can involve the α-granules or the dense (δ) granules. α-granules contain fibrinogen, factor V, thrombospondin, platelet-derived growth factor (PDGF), and vWF. The δ granules contain ADP, ATP, calcium, and serotonin, giving them an intrinsic electron density and dark appearance under electron microscopy (EM).

Defects in α-granules lead to the gray platelet syndrome (GPS) and the Quebec platelet disorder (QPD). GPS is an autosomal recessive disorder characterized by gray-appearing platelets, devoid of the red-staining α-granules on a peripheral smear. Electron microscopy corroborates the absence of α-granules (Nurden and Nurden 2011). Patients usually have normal to somewhat decreased platelet counts with mild bleeding and often present with splenomegaly and early-onset myelofibrosis. A variant of GPS, Medich giant platelet disorder, has also been described, where in addition to a decrease in α-granules the platelets contain membranous inclusions resembling cigars or scrolls White el al (2004).

QPD, on the other hand, has autosomal dominant inheritance with high penetrance. This disorder is characterized by large amounts of urokinase plasminogen activator (uPA) leading to increased conversion of stored platelet plasminogen to plasmin resulting in abnormal proteolysis of proteins stored in α-granules (Nurden and Nurden 2011). Genetic analysis has identified tandem duplication of PLAU, the uPA gene, as the underlying defect in QPD (Paterson et al. 2010). Patients present with normal to slightly low platelet count and delayed bleeding following invasive surgical procedures; anti-fibrinolytics such as ε-aminocaproic acid or tranexamic acid can be used to ameliorate their symptoms (Hayward and Rivard 2011).

Deficiency in dense granules leads to inherited syndromes such as Hermansky–Pudlak and Chediak–Higashi. Hermansky–Pudlak, an autosomal recessive syndrome due to mutations in HPS genes, is associated with oculocutaneous albinism, colitis, and pulmonary fibrosis in addition to a dense granule storage pool disease. Chediak–Higashi disease, a rare autosomal recessive disease due to mutation in the lysosomal trafficking regulator (LYST), is characterized by mild bleeding due to storage pool deficiency, recurrent infections, oculocutaneous albinism, neurological defects, and recurrent infections.

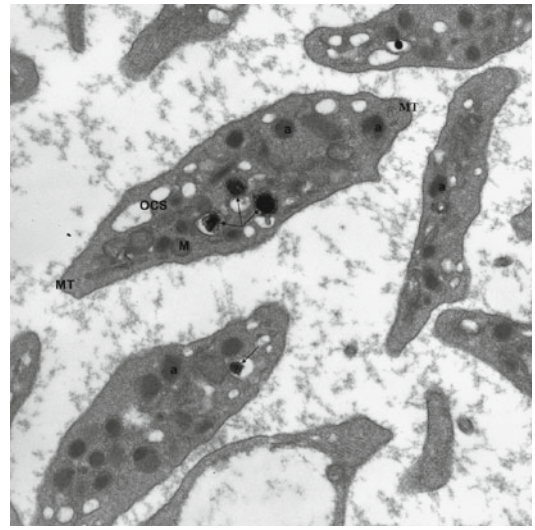

Fig. 6.2 Transmission electron microscopy of thin section of platelets: Several delta granules (*arrows*) are seen. Alpha granules (a) are observed. Microtubules (MT) are seen in cross section at either end of the top platelet. Mitochondria (M) and the open canalicular system (OCS) are present. Uranyl acetate lead citrate, ×30,000. *Photo courtesy of Dr. Tibor Nadasdy and Edward Calomeni*

These aforesaid dense granule diseases usually have normal coagulation tests. Platelet function testing should involve light transmission aggregometry (LTA) and EM. An EM picture of dense granule disease is shown below (Fig. 6.2).

Scott Syndrome

Scott syndrome, a very rare bleeding disorder, occurs due to a defect in the translocation of phosphatidyl serine from the inner to the outer platelet membrane. This hinders the binding of factors Va and Xa to the platelet membrane resulting in

impaired thrombin formation from prothrombin. Bleeding episodes are usually mild to moderate. Recently mutations in the splice-acceptor site of the TMEM16F gene have been reported to be associated with this entity (Suzuki et al. 2010).

Going back to the previous case, given her history of mucosal type bleeding, and bleeding after the surgical procedure that she did not receive DDAVP, there was a high level of clinical suspicion for an underlying coagulation disorder. The PT, PTT, and repeat vWD disease studies were normal in this patient. Additionally, a PFA was normal, but formal platelet aggregation studies were abnormal, demonstrating an absent secondary wave of platelet aggregation. Platelet electron microscopy studies were also consistent and showed a decreased number of dense granules (3.5 dense granules per platelet, range 4.0–6.0). These laboratory data were consistent with a diagnosis of a platelet storage pool disorder.

Drug-Associated Platelet Dysfunction

A 62-year-old female was referred for evaluation after having what was thought to be excessive bleeding after a minor dermatologic procedure. Previous pregnancies and surgical procedures in the past have been uneventful and without excessive bleeding. Her past medical history is notable for coronary artery disease for which she takes a baby aspirin daily and a recent diagnosis of depression for which she was started on a selective serotonin reuptake inhibitor. Her physical exam was remarkable only for multiple ecchymoses on her arm. Her CBC was normal, and both the PT and PTT were found to be normal.

Medications are a common cause of bleeding diatheses and are often overlooked due to an apparent lack of effect on coagulation testing. Warfarin affects vitamin K-dependent coagulation factors, so changes in PT and INR are expected. However, other agents can cause functional defects in the components of coagulation, without affecting the screening tests used for clinical evaluation. Commonly used agents that affect clotting include antiplatelet agents such as aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs), antidepressants, and herbal supplements.

Aspirin along with NSAIDs is one of the most commonly used drugs, and its use is facilitated by its ease of availability. Aspirin, the most commonly used antiplatelet agent, acetylates cyclooxygenases [greater cyclooxygenase-1 (COX-1) inhibition] irreversibly, thus preventing thromboxane A2 synthesis. By inhibiting prostanoid synthesis they inhibit platelet aggregation, leading to an increased bleeding risk. While the bleeding risk may be small particularly at low doses, the effect is often accentuated by the combined use of other antiplatelet agents such as clopidogrel or vitamin K antagonists such as warfarin. NSAIDs reversibly inhibit COX-1, generally to a lesser degree than aspirin. These agents are associated with increased bleeding due to inhibition of platelet aggregation. COX-2-selective NSAIDs do not appear to modify platelet activity and have a decreased bleeding risk, particularly gastrointestinal.

Clopidogrel and prasugrel are thienopyridines that work as ADP antagonists by inhibiting P2Y12 receptors, thus inhibiting platelet aggregation. While effective in reducing the risk of cardiovascular thrombosis, these agents augment bleeding risk due to inhibition of platelet function. However, these agents do not cause complete platelet antagonism. On the other hand, GPIIb/IIIa receptor antagonists such as abciximab and eptifibatide can cause severe bleeding due to irreversible inhibition of platelet aggregation.

Selective serotonin reuptake inhibitors (SSRIs) are among the most commonly prescribed agents for the treatment of depression. In addition to blocking the reuptake of serotonin in neurons, they also block reuptake in platelets

(Serebruany et al. 2003). The latter effect depletes intracellular stores of serotonin resulting in inhibition of aggregation of platelets and an increased risk of bleeding. While bleeding associated with SSRIs alone is often mild, the bleeding risk may be augmented when used in combination with antiplatelet and nonsteroidal anti-inflammatory agents (Labos et al. 2011; de Abajo and Garcia-Rodriguez 2008). Valproic acid, a commonly used antiepileptic agent commonly known to cause thrombocytopenia, can also cause increased bleeding with normal platelet counts. This is often due to abnormal platelet function and acquired vWD (Acharya and Bussel 2000). These effects are often reversed with dose reduction or cessation of the drug.

Many patients rely on herbal and vitamin supplements to help promote their general well-being. Since these agents are not FDA regulated or extensively studied, their mechanisms of action and adverse effects are often poorly characterized. Without a detailed and accurate history, the use of herbal supplements is often missed. Some of the commonly used herbal supplements such as garlic and ginkgo biloba among others can increase bleeding risk without any associated abnormalities in the coagulation function tests (Stanger et al. 2012). These agents may promote bleeding by inhibiting platelet aggregation or affecting coagulation factor levels. Moreover, these agents can potentiate the activity of other antiplatelet agents such as aspirin or indomethacin leading to increased bleeding. Also, commonly used supplements such as fish oil or vitamin E have been shown to cause bleeding tendencies in higher doses.

After a complete history and examination of the patient, it became increasingly clear that her post-procedure bleeding and increased ecchymoses may be related to the use of both aspirin and the SSRI. While SSRIs alone may not impair platelet function to the point that patients experience clinically relevant bleeding complications, their use in concert with other medications that impair platelet function such as aspirin can lead to clinically significant impairment of platelet function. The absence of bleeding complication after previous hemostatic challenges argues against a congenital platelet function abnormality.

Clotting Factor Abnormalities

A 25-year-old woman is evaluated at a hematology clinic. She recently underwent a routine tooth extraction and reported what she felt was increased bleeding 5 h later after the procedure. Her past medical history was unremarkable with no other surgical procedures, but she did report occasional nosebleeds and heavy menstrual cycles for as long as she can remember. Physical examination was unremarkable.

While deficiency of coagulation factors is expected to manifest an abnormality in the PT or the aPTT, many times factor levels may not be sufficiently low to produce alterations in these coagulation tests. Patients with such factor deficiencies usually have mild, if any, bleeding and are often discovered at the time of surgery. Typically, alterations in PT or aPTT are observed when the factors are less than 50 % of normal. There are three major clotting factor deficiencies or defects that are associated with bleeding diathesis but with normal coagulation tests. These include factor XIII deficiency, milder forms of vWD, and fibrinolytic pathway defects.

Factor XIII Deficiency

Factor XIII (fibrin-stabilizing factor) is a transglutaminase that circulates in the plasma as a tetramer of two catalytic A subunits and two carrier B subunits (Nourbakhsh et al. 2011). Activated factor XIII is formed in the presence of calcium

and fibrin catalyzed by thrombin. Factor XIIIa cross-links fibrin monomers to form a meshwork that leads to stabilization of the clot. Congenital factor XIII deficiency is inherited as an autosomal recessive disease and can involve either the A or the B subunits. Mutations in subunit A (type 2 defect) cause severe bleeding and usually manifest as umbilical cord bleeding in neonates, whereas mutations in subunit B (type 1 defect) cause mild bleeding and are often subclinical (Hsieh and Nugent 2008). Homozygous patients are characterized by intracranial hemorrhage, ecchymosis, and prolonged bleeding after injury or surgery. Poor wound healing and excessive scar formation are also seen in some patients. However, spontaneous bleeding does not usually occur in patients with factor XIII levels greater than 3–5 %.

Acquired factor XIII deficiency can also occur in liver disease, leukemia, myelodysplastic syndrome, sepsis, and disseminated intravascular coagulation. Additionally, autoantibodies against factor XIII can bind to plasma factor XIII interfering with its normal function. Standard laboratory tests to assess clotting such as platelet count, bleeding time, PT, and aPTT are typically within normal limits in patients with factor XIII deficiency. Therefore, patients with persistent bleeding symptoms and normal coagulation tests should be evaluated for factor XIII deficiency. The diagnosis is established based on increased solubility of the clot in 1 % monochloroacetic acid or 5 M urea, indicating clot instability and factor XIII deficiency (Hsieh and Nugent 2008). An abnormal solubility test indicates that the activity of factor XIII is less than 10 %. It is important to know that patients with alpha-2 antiplasmin deficiency can also demonstrate clot instability (see below). Factor XIII antigen levels and activity are measured using ELISA and photometric assays, respectively.

Treatment involves administration of cryoprecipitate and fresh frozen plasma, and prophylactic administration of these agents is used in patients with a prior history of intracerebral hemorrhage. Recombinant factor XIII-A2 has been shown to be a potentially effective alternative for factor XIII replacement in patients with congenital factor XIII deficiency (Lovejoy et al. 2006). On the other hand, patients with acquired factor XIII deficiency due to autoantibodies are treated with steroids, immunoglobulins, or rituximab (off-label use).

von Willebrand Disease

vWD is the most common and best-characterized primary hemostatic disorder with an estimated prevalence of about 1 % (Bowman et al. 2010). It can be categorized into three major forms: inherited, acquired, and platelet type. Inherited forms of vWD can be due to a qualitative or a quantitative deficiency of vWF, a multimeric protein that is required for platelet adhesion. While abnormalities in intrinsic pathway and thus alteration in aPTT are expected with vWD, milder forms can have a normal PT and aPTT, with an increased bleeding time. Type 1 vWD, which is due to a heterozygous quantitative deficiency of vWF, is the mildest and most common form of vWD and accounts for approximately 75 % of patients with vWD. Type 2 vWD is due to qualitative defects in vWF, and type 3 vWD is due to a homozygous, quantitative deficiency in vWF. Patients usually present with bleeding involving the skin and mucous membranes, with menorrhagia as a common initial presentation in women. Also, while a deficiency of factor VIII could lead to an abnormal aPTT, patients with mild hemophilia with greater than 30–40 % of the mean normal concentration of factor VIII would have normal or near-normal aPTT.

Testing for vWD should include vWF-Ag plasma levels, factor VIII assay as vWF acts as a carrier protein for factor VIII in plasma, and the ristocetin cofactor assay. It is important to note that since vWF is an acute-phase reactant, it can increase in response to stress and pregnancy. Additionally, estrogen can increase the synthesis of vWF and can similarly normalize vWD testing. Therefore, normal levels do not necessarily exclude a diagnosis in patients with history of bleeding diathesis, and at least two sets of labs should be performed. Treatments include DDAVP and factor concentrates that may con-

tain both vWF and factor VIII. DDAVP promotes the secretion of stored vWF from the endothelial cells and can be used to achieve hemostasis prior to surgery. Hyponatremia and seizures are severe side effects associated with DDAVP use; therefore, close monitoring of electrolytes and fluid restriction in the postoperative period is warranted. Plasma-derived concentrates such as cryoprecipitate, which contains more concentrated vWF/factor VIII, can also be used.

Fibrinolytic Pathway Defects

Alpha-2 Antiplasmin

While defects in coagulation cascade find a prominent role in the evaluation of bleeding diathesis, fibrinolytic pathways are often overlooked, as they are extremely rare. Fibrinolysis ensures the resolution of clots that are formed in response to tissue injury. To prevent excess bleeding and tissue damage, this process is strictly regulated.

Alpha-2 antiplasmin is the primary inhibitor of plasminogen, and congenital deficiency of alpha-2 antiplasmin has been associated with increased bleeding due to increased fibrinolysis (Carpenter and Mathew 2008). Although very rare, it is important to keep this diagnosis in mind during evaluation of bleeding diathesis, as typical screening tests for coagulation are usually normal. Inherited as autosomal recessive, bleeding associated with alpha-2 antiplasmin deficiency is often delayed after trauma or surgical procedures. While homozygous individuals present with severe bleeding during childhood, heterozygous patients tend to have milder bleeding or asymptomatic and bleeding occurs with trauma, surgery, or dental extraction. An acquired deficiency can be seen in patients with severe renal or liver disease. The euglobulin lysis time (ELT) can be used for assessment of patients and is shorter in patients with alpha-2 antiplasmin deficiency.

Plasminogen Activator Inhibitor-1 Deficiency

Plasminogen activator inhibitor-1 (PAI-1) is an important component of the coagulation cascade. It regulates fibrinolysis by controlling degradation of thrombin, with a deficiency leading to an increased bleeding risk (Mehta and Shapiro 2008). Affected patients usually have mild to moderate bleeding symptoms such as epistaxis, menorrhagia, and delayed bleeding after trauma or surgical procedures. Spontaneous bleeding is usually rare. Making the diagnosis can be very challenging due to the lack of sensitive and standardized assays (Mehta and Shapiro 2008). While it is important to assay both antigen and activity levels for diagnosis, vWD and platelet function disorders should be ruled out prior to evaluation of PAI-1 deficiency. Anti-fibrinolytic agents (e.g., ε-aminocaproic acid or tranexamic acid) are the mainstay of therapy.

Routine coagulation studies were performed to evaluate her for a coagulation disorder. Given her history of epistaxis, heavy menstrual cycles, and excess bleeding after a tooth extraction, there was concern for an underlying coagulation disorder. Her CBC and PT were both found to be normal, but the PTT was slightly prolonged at 36 s (range, 24–34 s). Ristocetin cofactor activity was found to be mildly decreased at 35 % (40–200 %), vWF antigen was low at 40 % (50–180 %), and factor VIII levels were 40 % (75–220 %), consistent with a diagnosis of type I vWD.

Abnormal Vascular Fragility

Easy bruising is observed not only in cases of defects in clotting factors or platelet function but also in disorders that affect the integrity of blood vessels. Abnormalities in vascular fragility associated with conditions such as vasculitis, cryoglobulinemia, scurvy, and amyloidosis can present with increased bleeding but without any observed alterations in PT, PTT, or platelet counts. Spontaneous, recurrent, and easy bruising is usually the hallmark of these disorders. A wide range of symptoms, ranging from mucosal bleeding and subcutaneous hematomas to excess

menstrual bleeding can occur. Postsurgical bleeding is immediate and can be severe. Skin discoloration due to recurrent bleeding and subsequent hemosiderin deposition is present. Typically, symptoms associated with connective tissue disorders such as delayed wound healing, joint hypermobility, and hyperextensibility of skin can be observed.

Ehlers–Danlos syndrome is an inherited connective tissue disorder due to defects in synthesis of collagen. In addition to hyperextensibility and joint hypermobility, the disorder is characterized by increased vascular fragility leading to easy bruising. Laboratory studies such as PT, PTT, and bleeding time are usually normal. The Hess (or tourniquet test or Rumpel–Leede test) test can be used to diagnose abnormal vascular fragility. While no specific treatment is available, supportive interventions such as ascorbic acid, which helps to cross-link collagen fibrils, and DDAVP may improve bleeding symptoms (Rydz and James 2012).

Amyloidosis can cause an acquired factor X deficiency due to absorption of this factor by amyloid fibrils in the liver and spleen, leading to increased bleeding. Additionally, the increased vascular fragility in these patients can accentuate the bleeding diathesis. Treatment of the underlying amyloidosis may help ameliorate the bleeding symptoms. Interestingly, in these cases, factor Xa levels can be used as a surrogate for monitoring the response of amyloidosis to treatment.

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Diagnosing Thrombocytopenia in the Clinic

7

Samir M. Dalia and Benjamin Djulbegovic

Background

One of the most common incidental findings in the clinic is thrombocytopenia. With the multitude of blood tests run today many patients present to their physicians with either isolated thrombocytopenia or thrombocytopenia with anemia or leukopenia/leukocytosis. Some patients may be symptomatic and need hospitalization, while others will be asymptomatic and will require follow-up. The aim of this chapter is to help clinical practitioners work up thrombocytopenia in their clinic patients while learning when these patients should be admitted and/or referred to a hematologist.

The normal platelet count in adults ranges from 150,000 to 450,000/μL (some facilities have different normal ranges). Thrombocytopenia is defined as a platelet count less than 150,000/μL. Cases are considered mild if the platelet count is between 70,000 and 150,000/μL and severe if the platelet count is less than 20,000/μL (Buckley et al. 2000). Spontaneous bleeding is increased when the platelet count is acutely less than 10,000/μL and is considered a hematology emergency (Cines and Blanchette 2002; Veneri et al. 2009).

There are three major etiologies that can lead to thrombocytopenia in patients: (1) decreased platelet production; (2) increased platelet consumption; or (3) sequestration of platelets.

Decreased platelet production indicates either bone marrow suppression or primary bone marrow failure. Either process can be due to medications, radiation, alcohol use, neoplastic disorders infiltrating the bone marrow, vitamin deficiencies, infections, and congenital thrombocytopenias.

Increased platelet consumption indicates that platelets are being consumed faster than their 7–10-day average life-span. Alloimmune destruction from transfusions; autoimmune syndromes; disseminated intravascular coagulation; drug-induced thrombocytopenia; heparin-induced thrombocytopenia (HIT); infections; mechanical destruction; preeclampsia; hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome; or thrombotic thrombocytopenic purpura (TTP) and immune thrombocytopenic purpura (ITP) can lead to increased platelet consumption and can also cause bone marrow suppression leading to decreased platelet production.

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Sequestration of platelets is usually caused by hypersplenism and/or liver disease. There are other causes of thrombocytopenia which cannot be placed into these categories which include gestational thrombocytopenia, dilutional thrombocytopenia, and pseudothrombocytopenia.

Thrombocytopenia can also be evaluated as an isolated cytopenia versus being found in conjunction with leukopenia/leukocytosis or anemia. A systematic approach is needed in the work-up of thrombocytopenia. In an outpatient setting it is important to begin the workup by assessing if a patient is stable for outpatient workup or if they need to be in a hospital setting. Patients who have thrombocytopenia with mild to no symptoms can generally be worked up as an outpatient. These include patients with thrombocytopenia with platelet counts >10,000/μL, minor bleeding such as epistaxis that can be easily controlled, or other non-life-threatening bleeding. In situations where a patient requires more immediate attention, with life-threatening bleeding, or if they are at risk for spontaneous bleeding (platelet count <10,000/μL) immediate hospitalization is recommended to monitor and stabilize the patient (Fig. 7.1).

The approach to a patient with thrombocytopenia is based on the combination of assessment of the likelihood of the underlying etiology and the importance/severity of the underlying condition. It is mostly based on pseudo-physiologic reasoning. Empirical evidence supporting the outline strategies is lacking.

Clinical Vignette 1

A 40-year-old female presents to her primary care doctor after life insurance blood work showed an isolated thrombocytopenia with a platelet count of 90,000/μL. Large clumps of platelets were reported on her peripheral smear. The patient denied any recent illnesses, medication use, other medical problems, or bleeding or bruising. The patient is wondering if there is anything to be concerned about.

Diagnosis

Initially it is important to differentiate between isolated thrombocytopenia and thrombocytopenia with anemia or leukopenia/leukocytosis. In patients with thrombocytopenia with other blood line changes, hematology consultation may be required sooner than in those with isolated thrombocytopenia.

History

In order to treat our patient in the clinical vignette, the most important step in obtaining a diagnosis is to perform a complete history and physical exam of the patient. History should include questions about bleeding, bruising, petechiae, melena, rashes, fevers, or recent infections. Medication history should be reviewed in detail, and specific questions about over-the-counter medications, supplements including quinine, and herbal products should be discussed since many of these can cause thrombocytopenia (Table 7.1). Old laboratory values (was she always thrombocytopenic?), history of immunizations, recent travel, transfusion history, travel history, family history of bleeding or blood disorders, and any recent hospitalizations should be reviewed. Recent hospitalizations may indicate heparin exposure and the possibility of HIT. Acute and chronic alcohol use history, potential HIV exposure, and possible pregnancy should also be part of the history (Veneri et al. 2009; Achterbergh et al. 2012; Gauer and Braun 2012).

Physical Exam

Physical exam should focus on evaluating for changes consistent with thrombocytopenia or life-threatening bleeding. This includes a complete neurological exam to exclude deficits that may point towards intracranial bleeding; a fundoscopic exam to evaluate for retinal hemorrhage which is suggestive of intracranial bleeding; oropharyngeal exam to look for gingival bleeding or wet purpura; an abdominal exam to evaluate for hepatosplenomegaly, bruising, or masses that could represent an underlying malignancy or hematoma; and lymph node exam to rule out

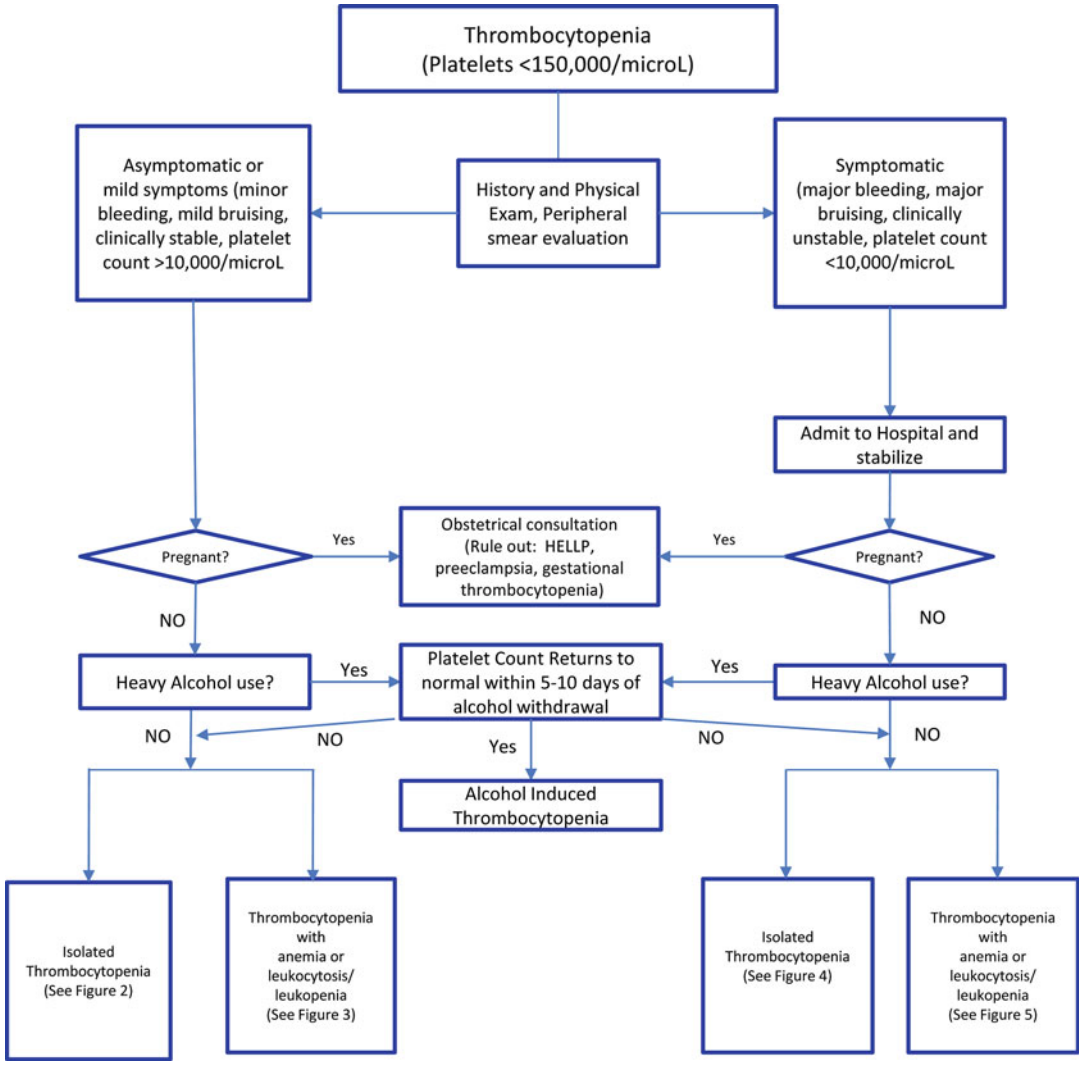

Fig. 7.1 Initial thrombocytopenia work-up

lymphadenopathy. Particular attention should be made towards a skin exam to look for petechiae, purpura, and bruising as well as assessment of epistaxis and mucosal, gastrointestinal, and genitourinary bleeding.

Laboratory Testing/Other Tests

A complete blood count (CBC), liver function testing, renal function testing, lactate dehydrogenase (LDH), and reticulocyte count are generally recommended in most patients presenting with thrombocytopenia. In patients presenting with thrombocytopenia alone, testing should be repeated in order to rule out pseudothrombocytopenia. A non-ethylenediaminetetraacetic acid (EDTA) anticoagulant such as citrate should be used for the repeat count to help differentiate pathologic from pseudothrombocytopenia which causes in vitro clumping caused by the EDTA. Other testing can be obtained based on the history and physical findings including bone marrow biopsy, HIV testing, hepatitis tests, and laboratory tests for autoimmune disorders. In patients with bleeding or who are hospitalized, prothrombin time (PT), partial thromboplastin time (PTT), fibrinogen, and fibrin split products can be checked in order to assess for a coagulation disorder. Peripheral blood smears should also be reviewed

Table 7.1 Non-chemotherapeutic agents commonly associated with thrombocytopenia. This table lists the most common drugs reported in the literature that have been shown to cause thrombocytopenia. For a more up-to-date database of drugs that cause thrombocytopenia check the reference database at http://www.ouhsc.edu/platelets

Abciximab	Hydrochlorothiazide
Alcohol	Interferon alpha
Acetaminophen	Methyldopa
Amiodarone	Naproxen
Ampicillin	Phenytoin
Carbamazepine	Piperacillin
Cimetidine	Procainamide
Chlorpropamide	Quindine
Cephalosporins	Quinine
Danazol	Ranitidine
Diclofenac	Rifampin
Eptifibatide	Sulfasalazine
Ethambutol	Trimethoprim/sulfamethoxazole
Gold salts	Simvastatin
Haloperidol	Valproic acid
Heparin (unfractionated and low molecular weight)	Vancomycin

in order to assess for platelet clumping (pseudothrombocytopenia), abnormalities in other blood lines including to assess for schistocytes which may indicate a hemolytic process, or abnormal blood cells such as leukemia cells.

Radiological testing can be obtained as indicated by history and physical exam. CT scanning of the abdomen can give the clinician information about the size of the spleen, and liver–spleen scanning (nuclear medicine) gives information about splenic activity (portal hypertension causes a colloid shift in this scan).

Figures 7.2–7.5 show algorithms for diagnosis of both asymptomatic and symptomatic thrombocytopenia.

Specific testing for disorders that present with thrombocytopenia is listed with each specific disorder.

Conditions and Treatment

Outpatient Conditions with Isolated Thrombocytopenia

Laboratory Error

There are multiple errors which may lead to a low platelet count. If the specimen is underanticoagulated or improperly drawn it can lead to small clots which result in thrombocytopenia. Secondly, blood samples may get mislabeled and could be reflecting thrombocytopenia on the wrong patient. There are also multiple technical errors that can occur in the laboratory which may result in thrombocytopenia.

CBC should be retested immediately in those cases of symptomatic thrombocytopenia and between 2 and 4 weeks in asymptomatic cases depending on the platelet count (Stasi et al. 2006).

Pseudothrombocytopenia

Pseudothrombocytopenia occurs when platelet clumping takes place in an EDTA-anticoagulated blood sample. This occurs in about 1 in 1,000 normal adults. Pseudothrombocytopenia can be ruled out with review of a peripheral blood smear showing platelet clumping. It has no clinical significance, and no further investigation is needed. A repeat platelet count can be done using an anticoagulant other than EDTA such as heparin or sodium citrate so that clumping does not occur (Onder et al. 1980; Bizzaro 1995; Froom and Barak 2011; Gauer and Braun 2012). Patients should be informed about their pseudothrombocytopenia and to tell other clinicians or hospitals that they should have blood counts done in non-EDTA anticoagulants.

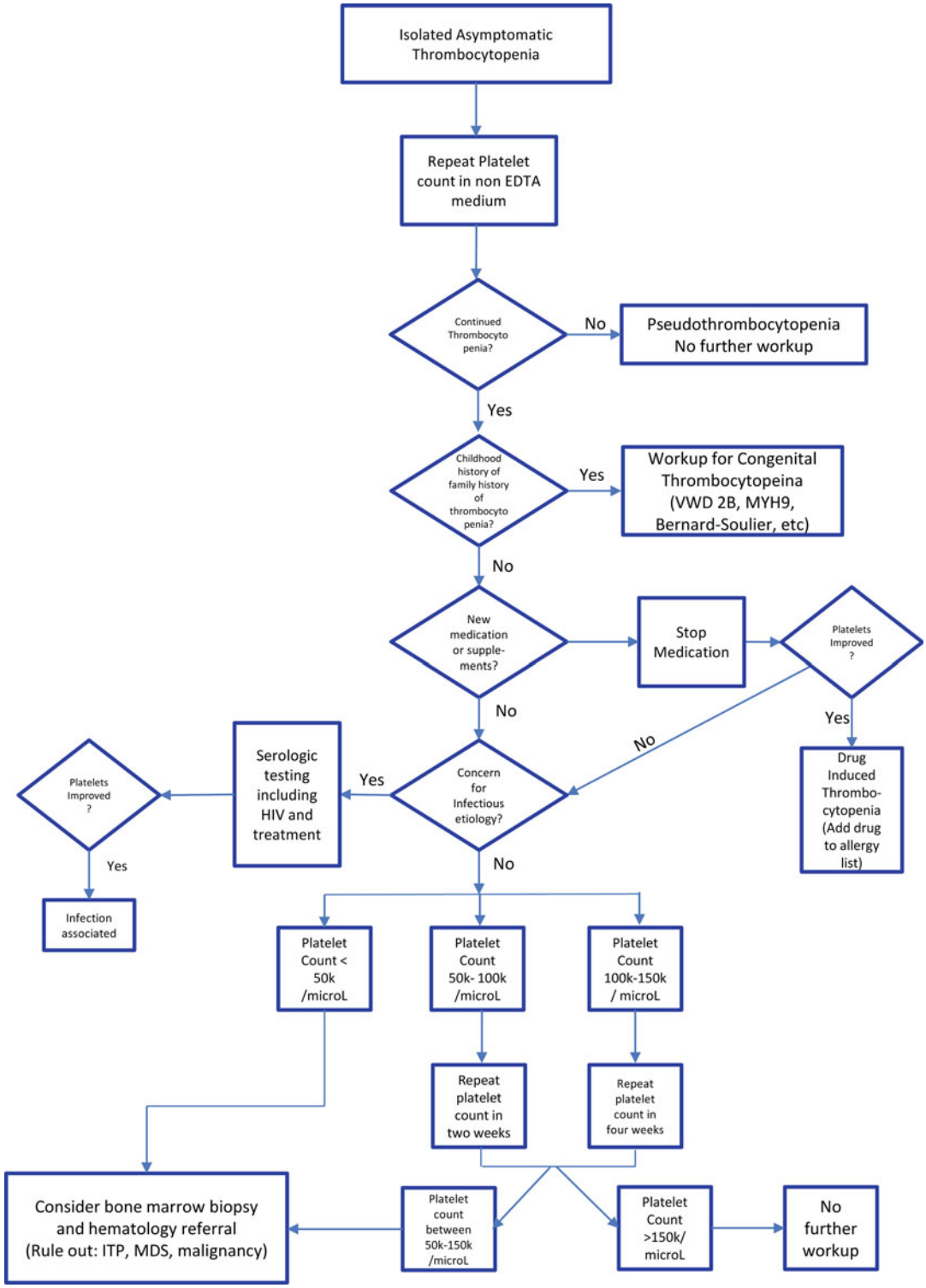

Fig. 7.2 Isolated asymptomatic thrombocytopenia work-up

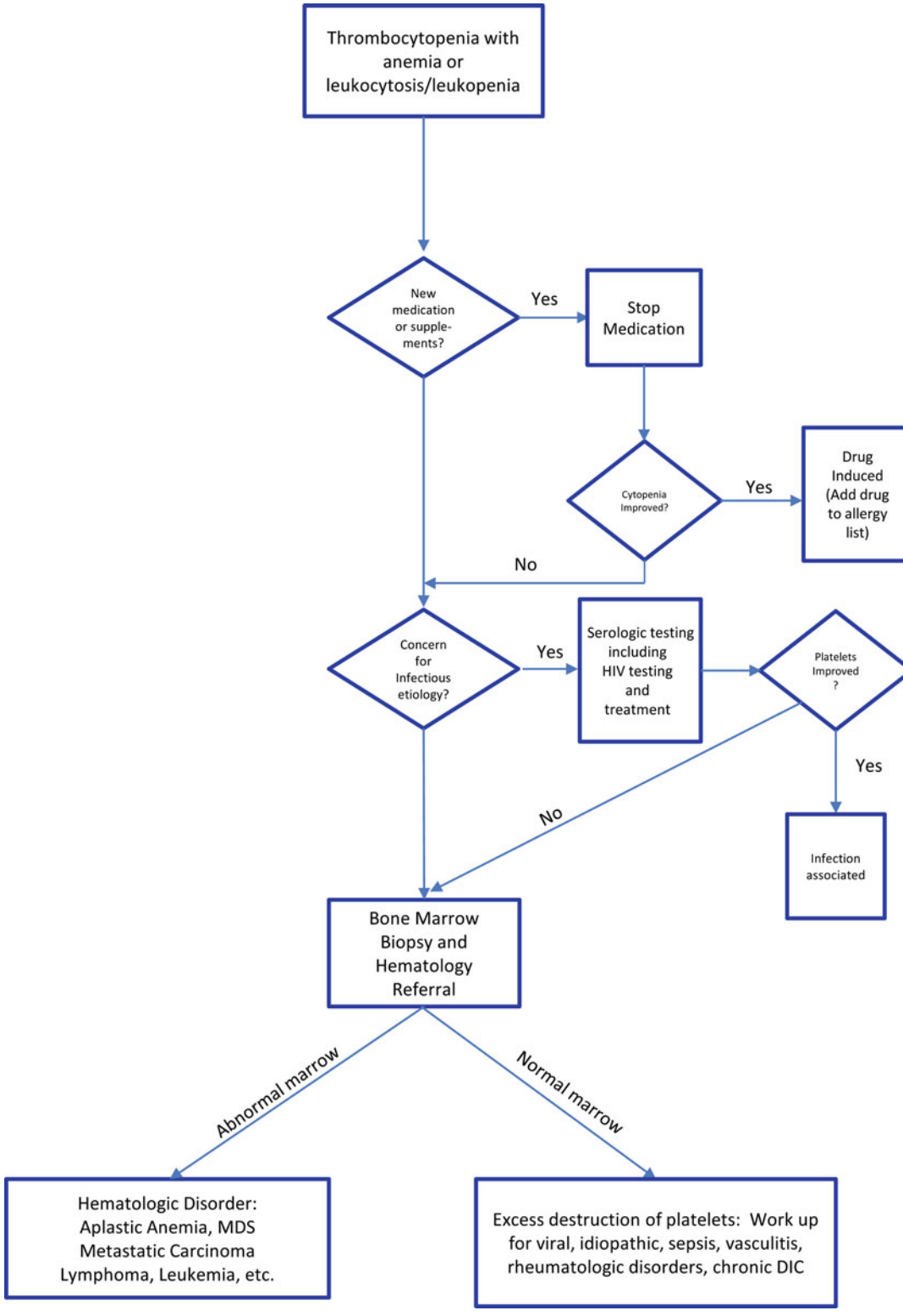

Fig. 7.3 Work-up for asymptomatic thrombocytopenia with anemia or leukocytosis/leukopenia

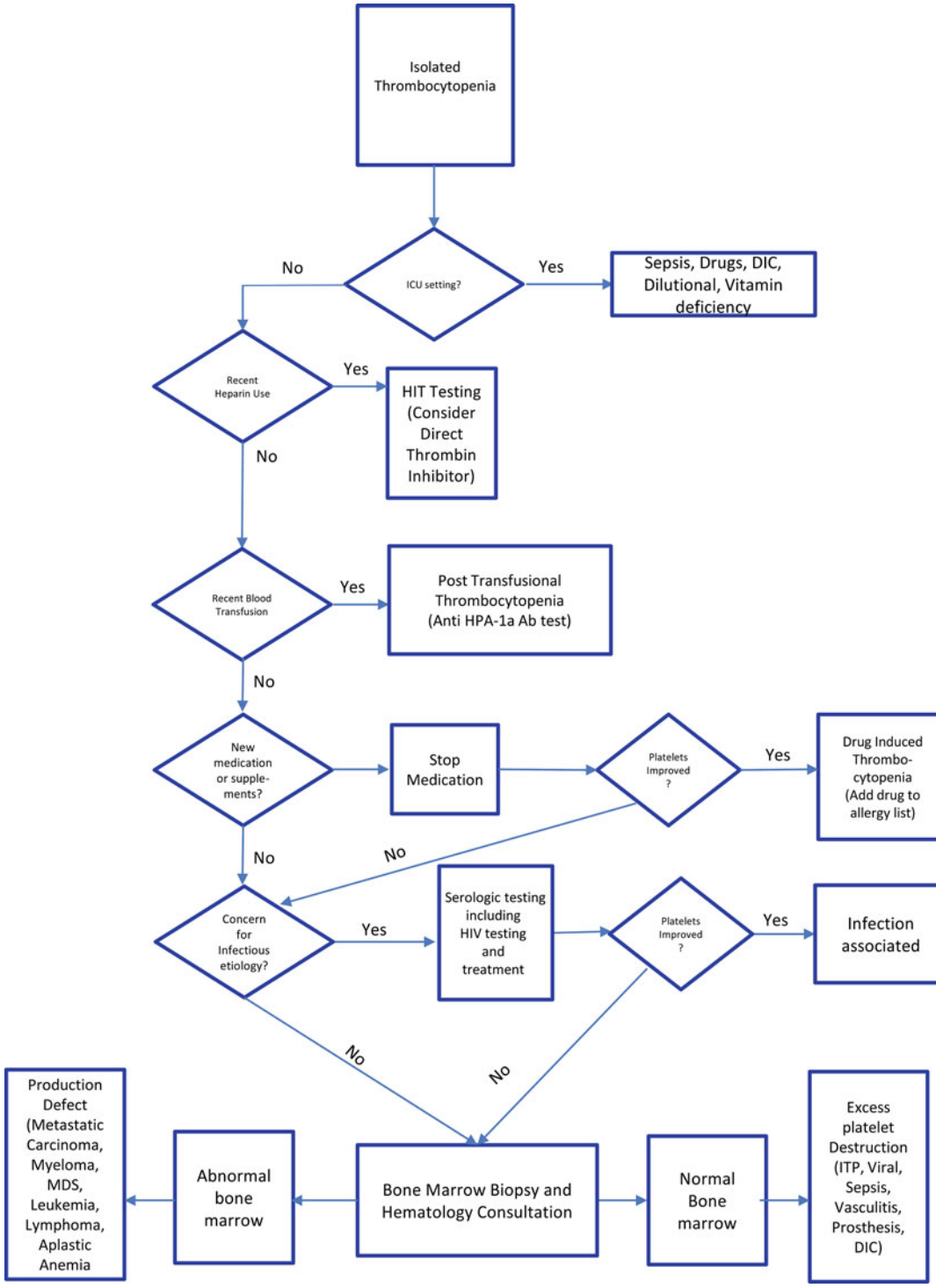

Fig. 7.4 Work-up for isolated symptomatic thrombocytopenia

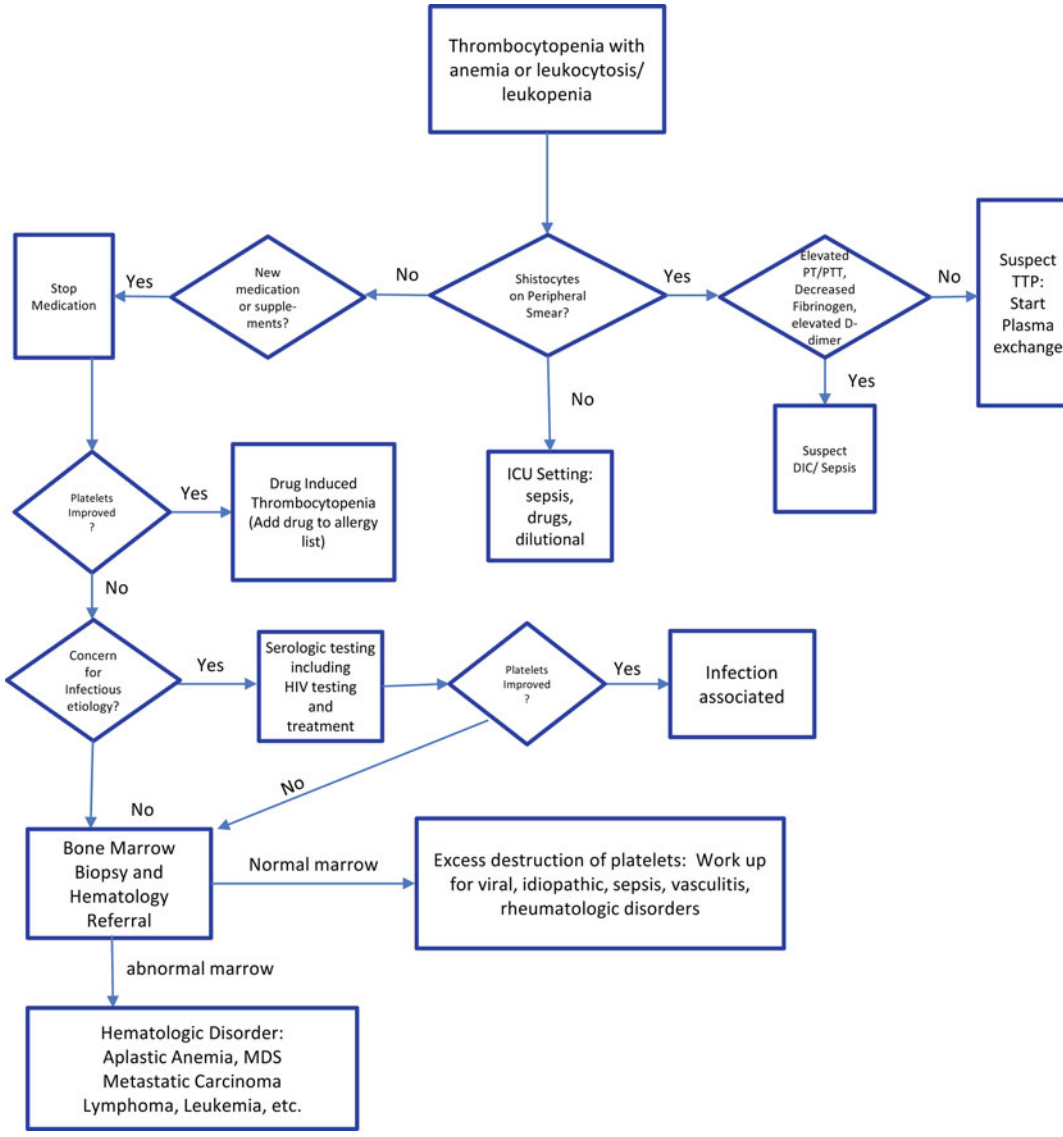

Fig. 7.5 Work-up for symptomatic thrombocytopenia with anemia or leukocytosis/leukopenia

Our 40-year-old had her platelets rechecked in a non-EDTA anticoagulant, and her platelet count was reported as 180,000/μL. The patient was alerted that future blood draws should be done in non-EDTA anticoagulants and that there was no other work-up or treatment required.

Dilutional Thrombocytopenia

Dilutional thrombocytopenia is usually seen in a hospital setting after hemorrhage or excessive fluid infusion. Platelet counts generally return to normal within 24–48 h after fluid shifts take place (Wong and Rose 2012). Even after as few as two units of blood transfusions, the platelet count can fall by half.

Gestational Thrombocytopenia

Five percent of women develop mild asymptomatic thrombocytopenia known as gestational thrombocytopenia. The etiology of this disorder is unknown, and it is clinically unimportant unless the platelet count is below 80,000/μL when there can be a concern for epidural anesthesia (van Veen et al. 2010). Thrombocytopenia should resolve following delivery and does not cause thrombocytopenia in the infant (McCrae et al. 1992; McCrae 2003, 2010).

Congenital Thrombocytopenia

Congenital disorders are usually associated with the presence of giant platelets. Congenital thrombocytopenia is often seen in a patient with a prolonged history of low platelets or if another family member also has a history of isolated thrombocytopenia. von Willebrand disease 2B (VWD 2B) and platelet-type VWD deserve mention since patients can often be asymptomatic and present in adulthood. Platelet-type VWD is a rare autosomal dominant disorder and is characterized by a defect in the glycoprotein 1 (GP1) receptor on the platelet membrane which increases its affinity to bind to the von Willebrand factor (vWF). Large platelet aggregates and high-molecular-weight vWF multimers are removed from the circulation resulting in thrombocytopenia. VWD 2B is a gain-of-function defect. GP1 receptor binding on the platelet membrane is abnormally enhanced leading to its spontaneous binding to platelets and rapid clearance of bound platelets and of the large vWF multimers. Thrombocytopenia can occur and is typically mild but can present clinically with excessive mucous membrane, menstrual, and/or postpartum hemorrhage (Sadler 2005; Nurden et al. 2009; Othman 2011). The diagnosis of 2B VWD is typically confirmed my performing ristocetin-induced platelet aggregation using low concentration of ristocetin or by molecular testing.

Peripheral blood smear may show pseudothrombocytopenia (clumping of platelets), and platelet size can be normal to large. In both platelet-type and VWD 2B, vWF antigen and the ristocetin cofactor (vWF activity) will be low. Factor VIII activity will be low, and the multimer pattern will be abnormal. In platelet-type VWD there is a mutation in GP1b. Desmopressin should be avoided as treatment as thrombocytopenia may worsen because of increased VWF multimers and increased platelet agglutination and clearance.

Other congenital disorders include MYH9-releated diseases (such as May–Hegglin disorder) and Bernard–Soulier syndrome (Wong and Rose 2012). MYH9-related thrombocytopenias are genetic conditions caused by mutations of the MHY9 gene. Platelets are large, and there is a low platelet count. There may be an associated hearing loss, cataracts, or renal insufficiency. The MHY9 disorders include May–Hegglin anomaly and Epstein, Fechtner, and Sebastian syndromes.

Posttransfusional Thrombocytopenia

Posttransfusional purpura should be suspected in patients who have an acute drop in platelets anytime up to 2 weeks after a blood transfusion. A typical presentation is an older, multiparous woman who presents with bleeding and severe thrombocytopenia after receiving blood products. Thrombocytopenia is due to recipient antibody to a platelet-specific antigen (usually HPA-1a) in patients who lack HPA-1a antigens. Diagnosis is made by the typical timing of a platelet drop after transfusion (usually hours to 2 weeks) and a positive anti-HPA-1a antibody.

Treatment is supportive, and high-dose immunoglobulins can be used in cases with severe thrombocytopenia or bleeding. Response is usually seen within 2–3 days in greater than 90 % of patients (Murphy and Bussel 2007; Zimring et al. 2011; Wong and Rose 2012).

Outpatient Conditions That Can Present with Multi-penias

Infectious Etiologies

Multiple viral and rickettsial infections can lead to thrombocytopenia usually with anemia or leukocytosis or leukopenia. Hepatitis C virus, cytomegalovirus, Epstein–Barr virus, and varicella zoster virus are all commonly associated with thrombocytopenia. Lyme disease, Rocky Mountain spotted fever, ehrlichiosis, and other tick-borne illnesses also can present with thrombocytopenia. Up to 10 % of initial presentation of

HIV cases are with thrombocytopenia (Franchini and Veneri 2006; Afdhal et al. 2008; Gauer and Braun 2012; Wong and Rose 2012). We recommend testing for HIV and hepatitis in all patients at high risk for these diseases who have thrombocytopenia and testing for other infectious etiologies based on history and geographic region.

Treatment of the underlying infection will correct the thrombocytopenia which may take weeks to return to a normal count.

Myelodysplastic Syndrome

In less than 10 % of cases myelodysplastic syndrome (MDS) can present with isolated thrombocytopenia. It is more common for patients to present with leukopenia or anemia in addition to thrombocytopenia (Wong and Rose 2012).

Liver Disease with Hypersplenism

Chronic liver disease with portal hypertension leading to congestive splenomegaly can typically present with thrombocytopenia with anemia or leukopenia. Platelet counts are usually above 50,000/μL, the spleen is typically enlarged on palpation, and in most patients liver function is affected resulting in a prolonged PT (McCrae 2003; Afdhal et al. 2008). Most patients do not have spontaneous bleeding, and liver transplant provides cure in patients that are eligible.

Alcohol Abuse

Thrombocytopenia is the most common hematologic abnormality in those who abuse alcohol. Alcohol ingestion leads to acute thrombocytopenia, and patients are commonly seen with low platelets after being admitted for binge drinking. Prolonged heavy alcohol consumption has a direct toxic effect on the bone marrow and causes of reversible suppression of platelet production, anemia, and leukopenia (Cowan 1980).

With alcohol cessation and adequate folate and thiamine repletion, recovery of platelet and other blood counts occur. Platelet counts usually improve in 1–2 weeks and can even initially present as a rebound thrombocytosis.

Autoimmune Conditions

Patients with autoimmune disorders including systemic lupus erythematous (SLE) and antiphospholipid antibody syndrome can also present with thrombocytopenia with other cytopenias. In SLE, an ITP-like syndrome can form in the setting of a multi-organ lupus flare. Patients can also have bleeding tendencies. Treatment with corticosteroids as in ITP is standard first-line therapy.

In patients with antiphospholipid antibody syndrome, thrombocytopenia is usually mild and sporadic. Since these patients have mild thrombocytopenia and are usually at risk for thrombosis, specific therapy is geared towards preventing thrombosis and not treating the underlying mild thrombocytopenia (Miller et al. 1983; Mader et al. 2002; Krause et al. 2005).

Hematologic and Solid Tumor Malignancies

Patients with malignancy can have thrombocytopenia with other cytopenias secondary to the disease or to medications. Chemotherapeutic agents used in treatment of malignancies can also lead to multiple cytopenias. Disorders such as acute leukemia, myelodysplastic syndrome, myelofibrosis, multiple myeloma, and lymphoma commonly present with multiple cytopenias. Diagnosis should include a bone marrow biopsy.

Treatment of the underlying disorder can lead to improvement in the cytopenias in cases where the malignancy is causing the cytopenias. Dose-reducing or -stopping chemotherapeutic agents resolve cytopenias in those induced by chemotherapy.

Clinical Vignette 2

A 60-year-old male with hypertension and diabetes presents to his primary care physician with a nose bleed and purpura on his arms and legs. The patient states that 2 days prior he was feeling fine. He states that he has not been sick and denies any other complaints. He has no fever or chills. CBC done in the office is normal except for a platelet count of 5,000/μL, and the patient presents with petechiae on his chest and back but no mucosal bleeding.

Drug-Induced Thrombocytopenia

Drug-induced thrombocytopenia is a common presentation of isolated symptomatic thrombocytopenia because a patient usually presents with bleeding caused from a sudden drop in platelet count. Patients with drug-induced thrombocytopenia can also present with anemia or leukopenia secondary to the drug use. This diagnosis should be excluded in all patients with thrombocytopenia.

Medications that were started within the past 4–6 weeks are more likely to be the cause of thrombocytopenia than those taken for many years. Drug-induced thrombocytopenia is usually caused by a drug-dependent antibody that is created by drug binding to a platelet surface protein.

Quinine is the most common medication that is associated with drug-induced thrombocytopenia (Nguyen et al. 2011). History of quinine use is not always given by patients since it is found in over-the-counter treatments for muscle cramps and also in tonic water. Other common medications implicated in drug-induced thrombocytopenia include abciximab, carbamazepine, heparin (either unfractionated or low-molecular-weight heparin), phenytoin, trimethoprim/sulfamethoxazole, and vancomycin. Heparin-induced thrombocytopenia will be discussed under symptomatic isolated thrombocytopenia. Table 7.1 includes a list of common medications that may cause drug-induced thrombocytopenia (Schiavotto et al. 1993; Rizvi et al. 1999; Arepally and Ortel 2006; George and Aster 2009; Nguyen et al. 2011).

Treatment is to stabilize the patient and continue to monitor the platelet counts. Inciting drugs should be removed and added to the patient's allergy list. A systematic review showed median recovery of platelets to be between 5 and 7 days. In this review of 266 patients, 23 (9 %) had major hemorrhage including two patients who died from bleeding (Rizvi et al. 1999; George and Aster 2009; Nguyen et al. 2011; Gauer and Braun 2012). Supportive inpatient care with platelets, packed red blood cells, and close monitoring are essential until platelet counts recover.

Preeclampsia

Preeclampsia can present with isolated thrombocytopenia. Neutrophilia can be present, and a mild hemolytic anemia is sometimes seen from shearing of red blood cells. Patients with severe preeclampsia with worsening elevations in blood pressure and/or worsening thrombocytopenia should be induced for delivery if possible. Expert consultation with an obstetrical specialist is needed to ensure proper care and potential delivery of the fetus (Baron and Baron 2005; McCrae 2010; Kadir and McLintock 2011). Treatment is delivery of the fetus with resolutions of symptoms within 1–2 days of delivery.

Primary Immune Thrombocytopenia

ITP is a diagnosis of exclusion in those patients with isolated thrombocytopenia. All other etiologies should be excluded prior to the diagnosis of ITP. No further evaluation outside of routine history, physical examination, CBC, and examination of the peripheral blood smear is needed. In patients older than 60 years of age, a myelodysplastic disorder is the more prevalent diagnosis and a bone marrow biopsy can be considered.

Treatment of ITP is complex and should be done in conjunction with a hematologist. ITP should only be treated if the patient is symptomatic and if their platelet counts are below 50,000/μL. Initial treatment is steroids. For further treatment guidelines please refer to the 2011 American Society of Hematology and 2010 International Consensus report on the treatment and diagnosis of ITP (Cines and Bussel 2005; Provan 2009; Provan et al. 2010; McCrae 2011; Neunert et al. 2011).

Our patient was admitted to the hospital with a hematology consultation, and after full work-up including a bone marrow biopsy to rule out myelodysplastic syndrome a diagnosis of ITP was made. The patient was started on corticosteroids and was monitored closely. He had no further bleeding, and his platelet count improved to around 40,000/μL in 6 days. The patient was discharged to continue treatment on an outpatient basis with his hematologist

Disseminated Intravascular Coagulation

Disseminated intravascular coagulation (DIC) is likely to present in a hospitalized setting and will be discussed further in another chapter in this book. DIC should be suspected in patients with an infection or a malignancy and abnormal PT and aPTT with a low fibrinogen and high-fibrin-split products. DIC is a medical emergency, and patients should be managed in a hospital setting.

Treating the underlying disorder will improve the thrombocytopenia. Supportive care including blood pressure support, fresh frozen plasma, packed red blood cells, antibiotics, and platelet transfusions are needed as indicated clinically (Taylor et al. 2001; Levi et al. 2009).

Heparin-Induced Thrombocytopenia

HIT should be suspected in patients with recent heparin exposures (up to 100 days). Platelet counts usually decline within 5–10 days after exposure to heparin and may decline within hours in patients with recent heparin exposure. Patients develop antibodies against platelet factor 4–heparin complexes causing platelet activation, thrombocytopenia, and thrombosis. Other characteristics include erythematous or necrotizing skin reactions at the site of injection, venous thrombosis, stroke, or myocardial infarction (Greinacher et al. 2005; Warkentin 2011).

A scoring system (4-T criteria) was developed utilizing the degree of thrombocytopenia, timing of thrombocytopenia after the initiation of heparin, thrombotic events, and exclusion of other causes of thrombocytopenia to aid in the diagnosis of HIT. In patients with a low score (0–3) HIT is unlikely. In those with a high score (6–8), HIT should be suspected and treatment should start immediately with a direct thrombin inhibitor. In those with an intermediate score (4–5) further testing is required to determine if the patient has HIT, but heparin should be discontinued and a direct thrombin inhibitor started until results are back (Lo et al. 2006). The most widely used test in suspected cases is an enzyme-linked immunosorbent assay with the platelet factor4/anion complex as the antigen (HIT ELISA). The test has 97 % sensitivity but a low specificity of 74–86 % (Arepally and Ortel 2006; Gauer and Braun 2012). A serotonin release assay can be used as a confirmatory test if there is a high suspicion of HIT, though in many institutions, it can take up to 7 days to result.

Treatment of HIT consists of immediately stopping any heparin products (unfractionated and low-molecular-weight heparin) including those given as in-line flushes. A heparin allergy should be added to the patient's medical record. A retrospective series of 62 patients with HIT showed that the 30-day risk of thrombosis was 53 % (Warkentin and Kelton 1996). For this reason patients should be started on a direct thrombin inhibitor (DTI) such as argatroban. Once the patient is stable on DTI therapy and their platelets are greater than 150,000/μL, the patient should be bridged to warfarin therapy. Anticoagulation should last at least between 2 and 3 months because of the high 30-day thrombosis risk, but no study has established the proper time course for anticoagulation (Warkentin 2011). HIT is a serious medical complication, and one study showed in-hospital mortality in patients who develop HIT to be as high as 20 % (Baroletti et al. 2008). HIT is discussed further later in this book (see Chap. 14).

Cardiac Surgery

Extracorporeal oxygenators lead to prolongation in the bleeding time and thrombocytopenia in patients who undergo cardiopulmonary bypass surgery. Platelet abnormalities are due to dilution by the priming solution and destruction of the platelets by the membrane of the machine. The platelet count usually drops by about 50 % and recovers within 4 days of surgery. HIT can also present in these patients as well and must be ruled out in any patients with cardiac surgery and a low platelet count (Wong and Rose 2012).

Thrombotic Microangiopathies: Thrombotic Thrombocytopenic Purpura/Hemolytic Uremic Syndrome

TTP/hemolytic uremic syndrome (HUS) are disorders that should be suspected in any patient presenting with microangiopathic hemolytic

anemia and thrombocytopenia. The classic pentad of fever, microangiopathic anemia, thrombocytopenia, renal disease, and neurological changes occurs in less than 20 % of cases. A diagnosis of TTP can be made with microangiopathic anemia (schistocytes seen on peripheral blood smear) and thrombocytopenia. Incidence of TTP is 4–11 patients/1 million annually in the United States (George 2009; George 2010). The condition is often caused by an absence or a deficiency of a disintegrin and metalloproteinase with thrombospondin type 1 motif, member 13 (ADAMTS 13) leading to ultra-long von Willebrand multimers that lead to microvascular aggregation of platelets resulting in ischemia and necrosis of tissue (Zheng et al. 2002; Sadler 2008; George 2010; Peyvandi et al. 2010). HUS is more likely to occur in children and can present with renal failure, bloody diarrhea, and/or abdominal pain. Shiga toxin-producing *Escherichia coli* is the most common causative organism in HUS (Hosler et al. 2003; George et al. 2008).

TTP is fatal without treatment. Patients should be admitted to the hospital if clinically suspected, and immediate daily plasma exchange should be started (Rock 2002; Michael et al. 2009; Nguyen and Han 2011). Corticosteroids and rituximab (off-label use) have also been used in the treatment of TTP, but plasma exchange remains the standard of care (George et al. 2006; Ling et al. 2009; George 2010). HUS in children related to infection can resolve on its own and may not require plasma exchange. Recently, eculizumab was shown to be effective in treatment of so called atypical HUS, which needs to be differentiate from a classic TTP (Legendre et al. 2010).

HELLP Syndrome

In patients who are pregnant with anemia, thrombocytopenia, and elevated liver enzymes the HELLP syndrome should be suspected. Many patients also present with right upper quadrant pain. Proteinuria is found with an elevated LDH.

Patients should be admitted and started on intravenous magnesium sulfate. Active management to deliver the fetus should be undertaken (McCrae et al. 1992; Martin et al. 2008; McCrae 2010; Gauer and Braun 2012).

Management of Patients with Chronic Thrombocytopenia

There is no consensus as to a time period when thrombocytopenic becomes chronic. In ITP, thrombocytopenia greater than 12 months is considered chronic ITP. Management of patients with chronic thrombocytopenia is aimed to prevent bleeding. In patients with thrombocytopenia and a multi-system disorder, the treatment of the primary disorder is the primary treatment goal. There is no evidence-based recommendations for a safe platelet count. Most experts agree that patients with platelet counts above >50,000/μL have levels adequate for hemostasis. Those with platelets between 30,000 and 50,000/μL rarely have purpura even with trauma. Those with platelet counts between 10,000 and 30,000/μL may be asymptomatic with everyday activities but can be at risk for excessive bleeding following more extensive trauma. Spontaneous bleeding typically does not occur unless platelet counts are <10,000/μL. Activity should only be restricted in patients with symptomatic thrombocytopenia or those with platelet counts <10,000/μL.

For invasive procedures, typically platelet counts greater than 40,000/μL provide safety for interventional procedures such as lumbar puncture, but this is controversial. In procedures at risk for complications even with minor bleeding such as neurosurgical procedures generally platelet counts greater than 80,000–100,000/μL are necessary. Commonly epidural anesthesia for obstetric delivery requires platelet counts of at least 80,000/μL (van Veen et al. 2010).

In patients with asymptomatic thrombocytopenia blood counts can be monitored monthly and then spaced out further as long as the platelet count is stable. In patients with symptomatic isolated thrombocytopenia, platelet counts should be checked daily to weekly depending on the causative reason until resolution of symptoms.

Thrombocytopenia in the clinic can become a challenge because of the many reasons that can lead to this laboratory finding. A good history and physical exam can help differentiate

life-threatening thrombocytopenia versus those cases that can be followed in the outpatient clinic. As more patients get complete blood counts on a routine basis it is essential that clinicians understand how to diagnosis and treat patients with thrombocytopenia.

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Thrombocytopenia in the Intensive Care Unit and After Solid Organ Transplantation

8

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Abbreviations

CMV	Cytomegalovirus
CPB	Cardiopulmonary bypass
DIC	Disseminated intravascular coagulation
DITP	Drug-induced immune thrombocytopenia
EBV	Epstein–Barr virus
ECMO	Extracorporeal membrane oxygenation
GVHD	Graft-versus-host disease
HHV	Human herpes virus
HIT	Heparin-induced thrombocytopenia
HPA	Human platelet antigen
HPS	Hemophagocytic syndrome
ICU	Intensive care unit
IL	Interleukin
ITP	Immune thrombocytopenia
PCR	Polymerase chain reaction
PTLD	Post-transplant lymphoproliferative disorder
PTP	Posttransfusion purpura
SOT	Solid organ transplantation
TMA	Thrombotic microangiopathy
VAD	Ventricular assist device
VZV	Varicella zoster virus

Introduction

Thrombocytopenia may arise from a diversity of mechanisms and etiologies (Table 8.1). In this chapter, we review the causes of, diagnostic approach to, and management of thrombocytopenia in two special populations: patients with critical illness and those who have undergone solid organ transplantation (SOT). Although such patients may be subject to any of the causes of thrombocytopenia that afflict individuals in other settings, herein we focus on etiologies of particularly high prevalence and/or clinical importance in the intensive care unit (ICU) and post-transplant settings.

Thrombocytopenia in the Intensive Care Unit

You are asked to urgently evaluate a 77-year-old woman with end-stage renal disease for the acute onset of severe thrombocytopenia and gastrointestinal bleeding. She was admitted to the hospital 10 days ago for methicillin-resistant *Staphylococcus aureus* bacteremia associated with her tunneled dialysis catheter. The catheter was replaced,

(continued)

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Table 8.1 Selected mechanisms and etiologies of thrombocytopenia

Pseudothrombocytopenia
Dilutional thrombocytopenia
Disorders of decreased platelet production
Congenital thrombocytopenias
Myelosuppressive therapy (e.g., radiation, chemotherapy)
Ethanol toxicity
Folate or vitamin B12 deficiency
Primary bone marrow disorders (e.g., myelofibrosis, myelodysplasia, leukemia)
Infiltrative diseases of the bone marrow
Certain viral infections (e.g., HIV, Epstein–Barr virus)
Hepatic insufficiency
Splenic sequestration
Portal hypertension
Infiltrative diseases of the spleen
Disorders of decreased platelet survival
Immune thrombocytopenia
Certain drugs (e.g., heparin, quinine)
Alloimmune thrombocytopenias (e.g., posttransfusion purpura)
Disseminated intravascular coagulation
Infection/sepsis
Thrombotic thrombocytopenic purpura/hemolytic uremic syndrome
Extracorporeal circuits and intravascular devices (e.g., cardiopulmonary bypass, intra-aortic balloon pump, ventricular assist device)

and she was initiated on vancomycin with clinical improvement and clearance of blood cultures. During this interval, her platelet count rose from 112×10^9/L on admission to 247×10^9/L when it was last checked 2 days ago. A red blood cell transfusion was administered 1 week ago. This morning, while awaiting discharge, the patient developed new-onset rectal bleeding and epistaxis. A repeat complete blood count showed a platelet count of 2×10^9/L. The prothrombin time and activated partial thromboplastin time were normal. You recommend immediate platelet transfusion and discontinuation of vancomycin and request a peripheral blood smear and testing for anti-human platelet antigen 1a antibodies.

Thrombocytopenia in the outpatient setting is discussed in Chap. 7. Thrombocytopenia in pregnancy is addressed in Chap. 17.

Epidemiology

Thrombocytopenia is traditionally defined as a platelet count less than 150×10^9/L, though some ICU studies have used lower cutoffs. Irrespective of definition, thrombocytopenia is common among patients with critical illness. In a systematic review of 24 studies, thrombocytopenia was present in 8.3–67.6 % of patients on admission to the ICU. Of those with a normal or a supranormal platelet count at admission, an additional 13.0–44.1 % of patients acquired thrombocytopenia during their ICU course (Hui et al. 2011). The large variation in the prevalence and incidence of thrombocytopenia among these studies is likely attributable to differences in patient population and definitions of thrombocytopenia.

Thrombocytopenia is more common in surgical than in medical ICU patients (Greinacher and Selleng 2010). Other independent risk factors for the development of thrombocytopenia include sepsis, organ dysfunction, and a high severity of illness (Hui et al. 2011; Greinacher and Selleng 2010) as measured by a variety of scales including the Acute Physiology and Chronic Health Evaluation (APACHE) score, Simplified Acute Physiology Score (SAPS), and Multiple Organ Dysfunction Score (MODS) (Vanderschueren et al. 2000; Strauss et al. 2002).

Clinical Manifestations

Bleeding

The most common clinical concern in patients with thrombocytopenia is bleeding. Among patients with immune thrombocytopenia (ITP), spontaneous bleeding is rare when the platelet count exceeds $20–30\times10^9$/L (Lacey and Penner 1977). An early study in patients with leukemia also suggested a relationship between bleeding risk and degree of thrombocytopenia (Gaydos et al. 1962). In a Cochrane review of studies

evaluating different platelet transfusion triggers in patients with hematologic malignancies, bleeding rates were similar with thresholds of 10×10^9/L or 20×10^9/L (Estcourt et al. 2012). A recent randomized controlled trial of different platelet doses in patients with chemotherapy-induced thrombocytopenia showed that major bleeding was primarily restricted to individuals with a platelet count of 5×10^9/L or less (Slichter et al. 2010). Therefore, in cancer patients, only severe thrombocytopenia (less than $5–10 \times 10^9$/L) is associated with a clear increased risk of bleeding (Arnold and Lim 2011).

Similar evidence of a relationship between platelet count and bleeding risk in the ICU is scant. Four studies have shown a significantly increased incidence of major bleeding in thrombocytopenic (27.1–39.0 %) compared to non-thrombocytopenic ICU patients (4.1–11.0 %) by univariate analysis (Vanderschueren et al. 2000; Strauss et al. 2002; Chakraverty et al. 1996; Ben Hamida et al. 2003). However, difference in risk between the two groups was no longer statistically significant after adjustment for confounders in the one study that applied multivariate analysis (Ben Hamida et al. 2003).

Based on the recognized importance of platelets in hemostasis, it is probable that thrombocytopenia below a certain threshold is associated with increased bleeding risk in ICU patients. What that threshold is and the magnitude of its contribution to bleeding risk require further study. Additional factors including the etiology of thrombocytopenia; the presence of congenital or acquired platelet dysfunction; coagulopathy due to liver disease, vitamin K deficiency, or hyperfibrinolysis; invasive procedures; acid–base disturbances; and hypothermia also contribute to an individual patient's bleeding risk (Greinacher and Selleng 2010).

Thrombosis

Thrombocytopenia is often used as a justification to withhold pharmacologic thromboprophylaxis in critically ill patients. However, thrombocytopenia cannot be presumed to be protective against thrombosis without an understanding of its etiology. Indeed, several relatively prevalent causes of thrombocytopenia in the ICU such as disseminated intravascular coagulation (DIC), heparin-induced thrombocytopenia (HIT), and postoperative state are associated with a *heightened* risk of thromboembolism. In an analysis of 408 patients with acute HIT, severity of thrombocytopenia was positively correlated with thrombotic risk (Greinacher et al. 2005).

Mortality

Whatever its cause, thrombocytopenia portends an ominous prognosis in patients with critical illness. At least six studies have shown an increased risk of in-ICU or in-hospital mortality in thrombocytopenic patients by multivariate analysis (OR 2.1–26.2) (Vanderschueren et al. 2000; Stephan et al. 1999b; Brogly et al. 2007; Martin et al. 2009; Vandijck et al. 2010; Caruso et al. 2010).

Select Causes

While critically ill patients are vulnerable to any of the multiplicitous acute and chronic causes of thrombocytopenia that afflict patients in other settings (Table 8.1), most cases of thrombocytopenia in the ICU are acute and arise around the time of or during admission to the ICU. In general, acute thrombocytopenic disorders affecting critically ill patients can be divided into two categories: those arising as a complication of the illness for which the patient is admitted (illness related) and those arising as a complication of management (iatrogenic) (Table 8.2). A brief description of each of these disorders is provided below.

Sepsis

Sepsis is an independent risk factor for thrombocytopenia in the ICU, and thrombocytopenia complicates 14.5–59.5 % of cases of sepsis (Martin et al. 2009; Vandijck et al. 2010; Charoo

Table 8.2 Common and clinically important causes of thrombocytopenia in the ICU

Illness-related causes	Iatrogenic causes
Sepsis	Dilutional thrombocytopenia
Disseminated intravascular coagulation	Drug-induced immune thrombocytopenia
	Heparin-induced thrombocytopenia
	Posttransfusion purpura
	Extracorporeal circuitry/ intravascular devices
	Surgery

ICU intensive care unit

et al. 2009; Lee et al. 1993; Sharma et al. 2007). Although the pathophysiology of sepsis-induced thrombocytopenia is not fully understood, platelet activation and consumption, peripheral immune destruction, marrow suppression, and hemodilution have been postulated (Hui et al. 2011; Kelton et al. 1979). Thrombocytopenia is usually mild or moderate unless a concomitant etiology such as DIC is present. The platelet count is lower in patients with severe sepsis and septic shock than in patients with sepsis without organ dysfunction or hypotension (Mavrommatis et al. 2000). Treatment involves supportive care and antimicrobial therapy.

Disseminated Intravascular Coagulation

DIC is a systemic consumptive thrombocytopenia and coagulopathy. It occurs not in isolation but as a sequela of an underlying disorder, the most common of which include sepsis, trauma and tissue injury, malignancy, and obstetrical complications. DIC complicates approximately 1 % of hospital admissions and a greater proportion of admissions to the ICU (Matsuda 1996). Clinical features include a propensity for both thrombosis due to activation of coagulation and hemorrhage due to depletion of platelets and clotting factors. Laboratory abnormalities of acute DIC include decreased fibrinogen and elevated fibrin degradation products (including D-dimers). Thrombocytopenia is characteristically moderate to severe and may be accompanied by microangiopathic changes on the peripheral blood smear. A platelet count of less than 100×10^9/L is observed in 50–60 % of patients with DIC, whereas 10–15 % of patients have a platelet count less than 50×10^9/L (Stephan et al. 1999a; Hanes et al. 1997). Treatment is aimed at the underlying disorder. Transfusion of platelets and coagulation factors is justified in patients who have major bleeding, have a high risk for bleeding, or require invasive procedures. Cautious use of heparin may be appropriate in select patients with thrombosis or refractory bleeding (Hook and Abrams 2012).

Dilutional Thrombocytopenia

Patients who receive large-volume resuscitation with packed red blood cells and/or intravenous fluids without concomitant platelet administration are at risk for dilutional thrombocytopenia. The degree of thrombocytopenia is related to the volume of fluid administered (Leslie and Toy 1991). In studies of massively transfused subjects, the platelet count ranged from 47 to 100×10^9/L and 25 to 61×10^9/L in patients receiving 15 and 20 units of red cells within a 24-h period, respectively (Leslie and Toy 1991; Counts et al. 1979).

Drug-Induced Immune Thrombocytopenia

The primary mechanism of drug-induced immune thrombocytopenia (DITP) is accelerated platelet destruction caused by drug- or drug metabolite-dependent antibodies (Aster et al. 2009). Antibodies may also target megakaryocytes, resulting in reduced platelet production (Perdomo et al. 2011). An extensive list of drugs has been implicated in DITP (Nguyen et al. 2011). An updated, evidence-based catalog of these agents is available at http://www.ouhsc.edu/platelets. Frequently reported drugs associated with DITP

Table 8.3 Frequently reported drugs associated with DITP

Class	Specific agents
Antibiotics	Beta-lactam antibiotics (especially piperacillin)
	Linezolid
	Rifampin
	Sulfonamides (especially trimethoprim–sulfamethoxazole)
	Vancomycin
Antiepileptics	Carbamazepine
	Phenytoin
	Valproic acid
Glycoprotein IIb/IIIa antagonists	Abciximab
	Eptifibatide
	Tirofiban
Miscellaneous	Gold compounds
	Heparin
	Quinidine
	Quinine

are shown in Table 8.3. Classically, the onset of thrombocytopenia occurs approximately 1–3 weeks after initial exposure to the offending agent (George et al. 1998; Pedersen-Bjergaard et al. 1997), though it may arise rapidly in patients with prior exposure and preformed antibodies. An exception to this rule is thrombocytopenia induced by glycoprotein IIb/IIIa antagonists, which may arise shortly after initial exposure due to the existence of naturally occurring antibodies (Bougie et al. 2002; Berkowitz et al. 1997). Thrombocytopenia in DITP is characteristically severe (median nadir platelet count 11×10^9/L) and is associated with major bleeding and fatal hemorrhage in 9 % and 1–4 % of cases, respectively. The median time to platelet recovery after withdrawal of the offending drug is 5–8 days (George et al. 1998; Pedersen-Bjergaard et al. 1997). Most patients require no specific therapy other than discontinuation of the offending agent. Although high-level evidence of efficacy is lacking, bleeding patients may also be treated with platelet transfusion, intravenous immune globulin, or corticosteroids (Pedersen-Bjergaard et al. 1997; Ray et al. 1990). Laboratory assays demonstrating drug-dependent antibodies may be useful for confirmation of the diagnosis (Aster et al. 2009) but are available only at select reference laboratories and do not yield results in a time frame necessary to inform initial clinical decision making.

Heparin-Induced Thrombocytopenia

HIT is a unique DITP caused by platelet-, endothelial-, and monocyte-activating antibodies against complexes of platelet factor 4 and heparin (Amiral et al. 1992). In contrast to most other forms of DITP, thrombocytopenia is relatively mild in HIT (median nadir platelet count $60–70 \times 10^9$/L), and the major clinical complication is thrombosis (venous and arterial) rather than hemorrhage (Warkentin 1998). Thromboembolism is present in approximately half of patients at the time of diagnosis (Greinacher et al. 1999), and an increased thrombotic risk persists for up to 30 days following discontinuation of heparin (Warkentin and Kelton 1996). In heparin-naïve patients, the platelet count begins to fall 5–14 days after initial heparin exposure. In patients with recent heparin exposure (usually within the last 30 days) and preformed HIT antibodies, the platelet count may fall immediately upon re-exposure (i.e., rapid-onset HIT) (Warkentin and Kelton 2001). Treatment requires cessation of heparin, initiation of an alternative anticoagulant, and avoidance or postponement of warfarin until platelet count recovery (Linkins et al. 2012; Cuker and Cines 2012). Laboratory testing is used to confirm the diagnosis. Immunoassays are highly sensitive but have poor positive predictive value due to detection of both platelet-activating and non-activating antibodies (Pouplard et al. 1999). Functional assays are more specific, but technical requirements preclude their use in all but a small number of reference laboratories (Sheridan et al. 1986). Owing to the prevalence of thrombocytopenia and thrombosis in the ICU, the limited specificity of widely available immunoassays, and the limited availability of more specific functional assays, HIT is frequently considered in patients with critical illness (Cuker and Cines 2012). Nevertheless, HIT is uncommon in the

ICU with an incidence of approximately 0.4 % (Selleng et al. 2008; Crowther et al. 2005; Crowther et al. 2010). Also see Chap. 14.

Posttransfusion Purpura

Posttransfusion purpura (PTP) is a rare transfusion reaction in which severe thrombocytopenia (typically less than 10×10^9/L), often lasting days to weeks, develops 5–10 days after transfusion of a platelet-containing product such as red cells or platelets (Mueller-Eckhardt, 1986). Patients with PTP have been sensitized to a foreign platelet antigen by pregnancy or prior transfusion. The antigen most commonly implicated is human platelet antigen-1a (HPA-1a) (Vogelsang et al. 1986). Antibody binding results in rapid clearance of antigen-positive transfused platelets. By a poorly understood mechanism, the recipient's antigen-negative autologous platelets are also destroyed. The diagnosis is confirmed by demonstration of a circulating alloantibody to a common platelet antigen (usually HPA-1a) in patient serum that is absent from the patient's platelets. First-line treatment is with intravenous immune globulin.

Extracorporeal Circuitry/ Intravascular Devices

Extracorporeal circuits such as cardiopulmonary bypass (CPB) and extracorporeal membrane oxygenation (ECMO) frequently result in thrombocytopenia due to platelet activation and consumption on artificial surfaces. In a study of 581 patients who underwent surgery on CPB, 56.3 and 2.9 % developed thrombocytopenia (less than 150×10^9/L) and severe thrombocytopenia (less than 50×10^9/L), respectively (Selleng et al. 2010). The platelet count falls by a mean of 40 % from preoperative levels after CPB (Nader et al. 1999), typically nadirs within 24–72 h after surgery, and begins to recover by postoperative day 4. ECMO induces a 40–50 % fall in platelet count within 1 h of initiation (Robinson et al. 1993; Cheung et al. 2000). Thrombocytopenia requiring platelet transfusion is nearly universal and persists until circulatory support is discontinued (Robinson et al. 1993). In addition to thrombocytopenia, CPB and ECMO are associated with acquired platelet dysfunction, which may exacerbate bleeding risk (Konkle 2011). Continuous renal replacement therapy, unlike CPB and ECMO, typically effects only minor, clinically insignificant reductions in platelet count (Mulder et al. 2003).

Indwelling intravascular devices may also reduce platelet counts. In a prospective study of 58 patients with acute coronary syndrome and insertion of an intra-aortic balloon pump, the platelet count decreased to a mean nadir of 63 % of the pre-insertion count by day 4, remained stable thereafter, and rose rapidly after pump removal (Vonderheide et al. 1998). Although thrombocytopenia is common among patients with ventricular assist devices (VADs) (Warkentin and Crowther 2007), the presence of a VAD was not associated with a significant reduction in platelet count in a controlled study (Steinlechner et al. 2009). VADs may predispose to bleeding through other mechanisms including platelet dysfunction and acquired von Willebrand disease (Steinlechner et al. 2009; Uriel et al. 2010). At least two studies have shown pulmonary artery catheters to be associated with thrombocytopenia in ICU patients (Bonfiglio et al. 1995; Vicente Rull et al. 1984), though a causative relationship has not been established.

Surgery

Major surgery, even in the absence of CBP, lowers platelet counts due to consumption, blood loss, and dilution. In a study of 1,415 admissions to a surgical ICU, the median nadir platelet count was 113×10^9/L on postoperative day 2 and typically recovered to preoperative levels by days 3–5 (Nijsten et al. 2000). The fall in circulating platelet count after surgery leads to an increase in thrombopoietin levels with consequent stimulation of megakaryopoiesis, resulting in a postoperative thrombocytosis that is often two- to threefold greater than the preoperative baseline and peaks

approximately 2 weeks after surgery (Warkentin et al. 2003; Kaushansky 2009). A blunted or an absent rise in platelet count beyond postoperative days 3–5 suggests the presence of another etiology of thrombocytopenia (e.g., sepsis) (Greinacher and Selleng 2010) and is associated with increased mortality (Nijsten et al. 2000).

Diagnostic Approach

Our approach to thrombocytopenia in the ICU is shown in Fig. 8.1. We make every effort to obtain prior platelet counts to confirm that the thrombocytopenia is acute and not reflective of a chronic thrombocytopenic disorder. In addition, we use information from the history including a detailed chronicle of exposure to drugs and transfusions; the physical examination and radiographic studies for evidence of bleeding or thrombosis; the severity and timing of onset of thrombocytopenia and the pace of fall in the platelet count over the patient's hospital course; and the peripheral blood smear to form our initial differential diagnosis. On the basis of this assessment, specialized laboratory testing is requested for specific entities that we suspect.

Several patterns of clinical presentation are worth highlighting. Immune-mediated thrombocytopenic disorders such as DITP, HIT, and PTP typically present with an abrupt fall in the platelet count 5–14 days after exposure to the offending drug or blood product. Whereas DITP and PTP are typified by severe thrombocytopenia and bleeding, HIT is characterized by moderate thrombocytopenia and predisposition to thromboembolism. Platelet counts that fall within 48 h of large-volume resuscitation, surgery, CPB, or insertion of a balloon pump are often due to the intervention, itself. Sepsis, DIC, and disorders of impaired platelet production characteristically manifest as more gradual declines in platelet count over 5–7 days. In practice, thrombocytopenia in the ICU is often multifactorial, and patients may not fit a single stereotypical pattern. Nevertheless, we find the algorithm shown in Fig. 8.1 to be a useful guide for the evaluation of these complex patients.

Management

Appropriate management is highly dependent on the etiology of thrombocytopenia. For instance, whereas platelet transfusion and suspension of anticoagulant prophylaxis may be indicated in a patient with severe DITP, HIT requires prompt initiation of an alternative anticoagulant in lieu of heparin and constitutes a relative contraindication to platelet transfusion. Treatment must therefore be individualized to the underlying cause of thrombocytopenia as well as any concomitant thrombotic or hemorrhagic risk factors the patient may harbor.

As noted, high-quality evidence linking a platelet count threshold with bleeding risk in ICU patients is lacking. If a patient with platelet dysfunction (congenital or acquired) has major bleeding, platelet transfusion is indicated irrespective of the platelet count. Published guidelines recommend a platelet count trigger of 50×10^9/L for prophylactic platelet administration in patients with DIC or massive transfusion and platelet counts of $50–100 \times 10^9$/L for invasive interventions, depending on the nature of the procedure (Samama et al. 2005; British Committee 2003; Practice Guidelines 1996; Practice Parameter 1994). These recommendations are based largely on expert opinion and experience rather than data. Higher quality evidence supports the practice of prophylactic platelet transfusion to maintain a platelet count of at least 10×10^9/L in non-bleeding oncology patients, whether in the ICU or on the wards.

It is also crucial to consider adjunctive measures for control of bleeding. These include replacement of deficient clotting factors (including fibrinogen); maintenance of a hemoglobin of 10 g/dL or more to optimize rheology for hemostasis (Valeri et al. 2001); use of antifibrinolytic agents (except in DIC); surgical, endoscopic, or interventional radiologic procedures to stop bleeding; and correction of uremia, acid/base disturbances, and hypothermia.

Observational data suggest that platelet transfusion is overused in the ICU (Blood Observational Study 2010). In a retrospective study of 76 platelet transfusions administered to

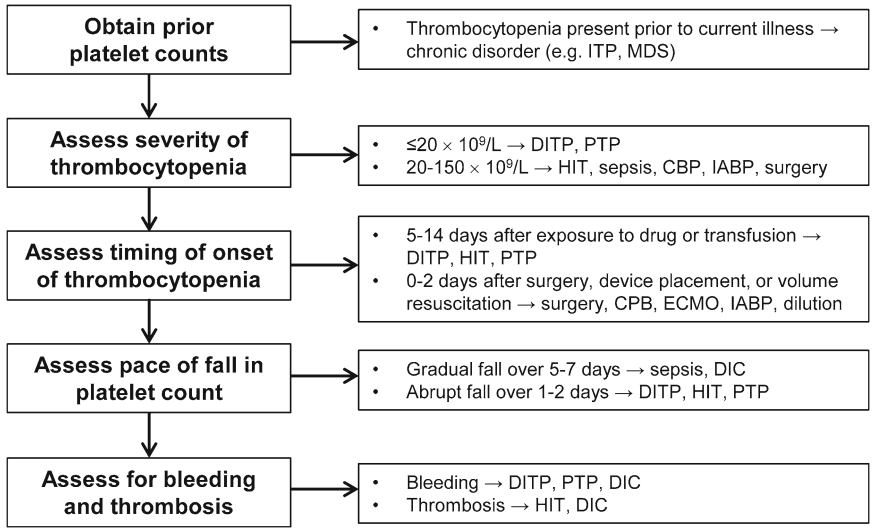

Fig. 8.1 An approach to the diagnosis of thrombocytopenia in the ICU. *ITP* immune thrombocytopenia, *MDS* myelodysplastic syndrome, *DITP* drug-induced immune thrombocytopenia, *PTP* posttransfusion purpura, *HIT* heparin-induced thrombocytopenia, *CPB* cardiopulmonary bypass, *IABP* intra-aortic balloon pump, *ECMO* extracorporeal membrane oxygenation, *DIC* disseminated intravascular coagulation

27 patients, the threshold platelet count for prophylactic transfusion was 33×10^9/L (Arnold et al. 2006). The same study indicated that use of liberal transfusion may be associated with an increased risk of infection, length of stay in the ICU, and mortality, though these observations remain to be validated in a controlled trial.

Thrombocytopenia among ICU patients may lead not only to platelet transfusion but also to delay of invasive procedures and withholding of anticoagulant thromboprophylaxis. Although high-quality evidence is not available, it has been proposed that prophylactic dose anticoagulation is likely to be safe in most patients with platelet counts of 30×10^9/L or greater. Higher platelet counts may be necessary for individuals with other risk factors for bleeding or those who require therapeutic dose anticoagulation (Arnold and Lim 2011). Randomized controlled trials are needed to guide platelet transfusion and anticoagulant thromboprophylaxis practice in thrombocytopenic patients in the ICU.

The peripheral blood smear shows severe thrombocytopenia without clumping, schistocytes, or other abnormalities. The patient's serum tests negative for HPA-1a antibodies. HPA-1a typing of her own platelets cannot be performed due to the severity of her thrombocytopenia. The patient receives intravenous immune globulin and platelet transfusions for suspected vancomycin-induced thrombocytopenia. She also undergoes hemodialysis with a high permeability filter to maximize drug removal (Castellano et al. 2008). Her platelet count normalizes 1 week after discontinuation of vancomycin. Vancomycin-dependent antiplatelet antibody testing is sent to a reference laboratory and is positive, confirming the diagnosis of DITP due to vancomycin. Vancomycin is added to the patient's list of allergies, and she is advised to obtain a Medic-alert bracelet.

Thrombocytopenia After Solid Organ Transplantation

You are asked to see a 36-year-old woman for pancytopenia. She underwent renal transplant 6 weeks ago for end-stage diabetic nephropathy. Over the last week, she has developed a rash and fever. Her laboratory

(continued)

studies demonstrate a white blood cell count of 2.0×10^9/L, a hemoglobin of 7.2 g/dL, and a platelet count of 38×10^9/L. She is taking azathioprine, prednisone, and tacrolimus. There have been no signs of graft rejection or dysfunction. Blood cultures are negative. The patient was cytomegalovirus (CMV) seronegative and received a cadaveric allograft from a CMV-seropositive donor. You recommend CMV nucleic acid testing, peripheral blood lymphocyte chimerism studies, and bone marrow aspirate and biopsy. You also recommend discontinuation of azathioprine.

Select Causes

Thrombocytopenia, either in isolation or in combination with other cytopenias, is a common finding among SOT patients and may be an early sign of a serious or a life-threatening disorder (Smith 2010). Although SOT patients are subject to any of the myriad causes of thrombocytopenia that affect other populations (Table 8.1), the discussion that follows is limited to etiologies of particular clinical relevance to the post-transplant setting. These etiologies include drug-, infection-, and immune-mediated complications of SOT (Table 8.4).

Table 8.4 Etiologies of thrombocytopenia after solid organ transplant

Category	Etiologies
Drug mediated	Drug-induced thrombocytopenia
	Calcineurin inhibitor-induced thrombotic microangiopathy
Infection mediated	Viral infection (e.g., CMV, EBV, VZV, HHV6)
	Infection-induced hemophagocytic syndrome
	Post-transplant lymphoproliferative disorder
Immune-mediated	Graft-versus-host disease
	Immune thrombocytopenia (ITP)

CMV cytomegalovirus, *EBV* Epstein–Barr virus, *VZV* Varicella zoster virus, *HHV6* human herpes virus-6

Drug-Induced Thrombocytopenia

SOT patients are routinely exposed to a variety of drugs that can cause cytopenias. Immunosuppressive and antimicrobial agents are frequent culprits. The mechanisms of drug-induced thrombocytopenia are varied and include marrow suppression and immune destruction (i.e., DITP). A third mechanism, thrombotic microangiopathy (TMA) due to calcineurin inhibitors, is discussed separately (see "Thrombotic Microangiopathy" below).

Perhaps the most common culprit in the SOT setting is the purine analog, azathioprine, which causes dose-related marrow suppression that may present as single or multilineage cytopenias. In a series of 739 inflammatory bowel disease patients treated with azathioprine, 37 (5 %) and 15 (2 %) developed clinically significant cytopenias and thrombocytopenia, respectively. Most cases arose during the first 4 weeks of treatment, but late presentations also occur (Connell et al. 1993). Because azathioprine and its principal metabolite are predominantly cleared in the kidneys, toxicity may occur in the setting of worsening renal function (e.g., in a renal transplant patient with allograft rejection). Thiopurine methyltransferase deficiency and concomitant use of several drugs commonly prescribed in the post-transplant setting such as angiotensin-converting enzyme inhibitors, allopurinol, and trimethoprim/sulfamethoxazole also increase drug levels and predispose to hematologic toxicity.

Other commonly employed immunosuppressive agents such as mycophenolate mofetil, tacrolimus, cyclosporine, and sirolimus may also cause cytopenias through suppression of hematopoiesis. Anti-thymocyte globulin is associated with the frequent occurrence of a mild, immune-mediated fall in the platelet count that typically resolves within a week of initiation of therapy (Rostaing et al. 2010). Alemtuzumab, a humanized anti-CD52 antibody that depletes T- and B-lymphocytes, is associated with a high incidence of thrombocytopenia within the first few weeks of therapy. An increased rate of ITP occurring months after exposure has also been reported (Cuker et al. 2011).

Ganciclovir and valganciclovir, which are used for prevention and treatment of CMV disease after SOT, cause marrow suppression and cytopenias. Cytopenias including thrombocytopenia are a common complication of treatment with trimethoprim–sulfamethoxazole and may result from DITP or interference with folic acid metabolism (Asmar et al. 1981).

Thrombotic Microangiopathy

TMA is characterized by intravascular platelet aggregation and thrombosis in the microcirculation, leading to microangiopathic hemolytic anemia, thrombocytopenia, and end-organ ischemic injury. Clinical and laboratory features include anemia, elevated lactate dehydrogenase, reticulocytosis, fragmented and nucleated erythrocytes in the peripheral blood smear, variable renal dysfunction, and multiorgan dysfunction in severe cases.

TMA in SOT patients most commonly arises as a complication of calcineurin inhibitor therapy. The disease occurs more frequently with cyclosporine but has also been reported with tacrolimus (Trimarchi et al. 1999). The addition of sirolimus to either drug appears to increase the risk (Hachem et al. 2006). The proposed mechanism is drug-induced direct endothelial injury (Myers 1986). In vitro studies suggest that cyclosporine may also enhance platelet aggregation (Grace et al. 1987). Histologic evidence of TMA is generally restricted to the kidneys (Zarifian et al. 1999). In a series of 950 kidney recipients, the incidence of calcineurin-induced TMA was 1.3 % and median onset was 7 days after transplant (Said et al. 2010). In most patients, the disease resolves with discontinuation of the offending agent. Although high-quality evidence to support its use in this setting is lacking, plasma exchange is often used in severe cases and those that do not resolve quickly after drug cessation. In renal transplant recipients, two other entities may cause a TMA-like picture and must be differentiated from calcineurin inhibitor-induced disease: hyperacute humoral rejection and administration of muromonab-CD3 (OKT3), an anti-CD3 monoclonal antibody given for prevention of acute rejection (Abramowicz et al. 1992).

TMA may also arise as a complication of the transplant, itself. This phenomenon, known as post-transplant TMA, is well documented following allogeneic hematopoietic stem cell transplantation, but reports following SOT in the absence of calcineurin inhibitor use are scant (Lipshutz et al. 2008). The pathogenesis of this disorder is not well understood. Proposed mechanisms include direct endothelial injury by immunosuppressive drugs, allograft rejection, post-transplant lymphoproliferative disorder (PTLD), and infection. In rare cases, severe acquired ADAMTS13 deficiency due to an inhibitor has been reported (Pham et al. 2002; Mal et al. 2006). Management involves withdrawal of any suspected immunosuppressive agents, evaluation for CMV and other viral infections, and supportive care. Plasma exchange may be attempted in severe cases but is probably of limited utility (George et al. 2004).

Viral Infections

Immunosuppressed SOT recipients are vulnerable to a multitude of opportunistic infections, a number of which may be associated with cytopenias. The most prevalent of these is CMV. Older literature cites an incidence of symptomatic CMV infection of 8, 29, 25, and 39 % in renal, liver, heart, and lung transplant patients, respectively (Patel et al. 1996). These rates have likely been reduced by modern prevention strategies (Andrews et al. 2011), but CMV disease remains common. Risk factors include type of transplant, intensity of immunosuppression, and CMV serologic status of the donor and recipient (with highest risk when a CMV-seronegative patient receives an organ from a CMV-seropositive donor). The characteristic clinical presentation consists of fever, arthralgias, myalgias, and cytopenias. Invasive tissue disease affecting the gut, lungs, retina, central nervous system, or allograft may also occur. In a series of 100 renal transplant patients with CMV disease, the incidence of thrombocytopenia was 43 % (Pour-Reza-Gholi et al. 2005). Proposed mechanisms of CMV-induced thrombocytopenia include direct infection of megakaryocytes or other hematopoietic

progenitors (Crapnell et al. 2000), immune destruction (Sahud and Bachelor 1978), or splenic sequestration (Sola-Visner et al. 2009). Current guidelines recommend quantitative polymerase chain reaction (PCR) viral load or antigenemia testing for diagnosis of CMV infection (Kotton et al. 2010). First-line agents for prevention and treatment of CMV disease are ganciclovir and valganciclovir (Andrews et al. 2011), which themselves may cause thrombocytopenia through myelosuppression.

Varicella zoster virus (VZV) and Epstein–Barr virus (EBV) are other herpes viruses that may cause thrombocytopenia in both immunocompetent and immunocompromised hosts (Carter 1965). Mechanisms include immune destruction (Steeper et al. 1989) and splenic sequestration. EBV viremia after SOT may also be associated with PTLD (discussed separately, see "Post-transplant Lymphoproliferative Disorder"). Human herpes virus-6 (HHV6) may present with cytopenias, fever, pneumonitis, hepatitis, and/or encephalitis after SOT (Dockrell and Paya 2001). Reactivation of HHV6 from latency is common in this setting, but clinical disease is probably infrequent (Hentrich et al. 2005) and may be difficult to distinguish from disease caused by other viruses such as CMV. The preferred method for diagnosing viral reactivation is quantitative PCR. First-line therapy is ganciclovir or foscarnet.

Infection-Induced Hemophagocytic Syndrome

Infection may also induce hemophagocytic syndrome (HPS), a life-threatening systemic inflammatory disease in which there is hemophagocytosis by activated, nonneoplastic macrophages in the bone marrow, lymph nodes, liver, and spleen. The disorder results from aberrant T-cell activation in response to a precipitating infection, leading to elaboration of macrophage-activating cytokines such as interleukin-2 (IL-2) and gamma-interferon. The activated macrophages, in turn, secrete the T-cell-activating cytokines IL-1, IL-6, IL-12, and tumor necrosis factor-α, producing a vicious cycle of T-cell and macrophage activation (Smith, 2010). Clinical and laboratory features include fever, hepatosplenomegaly, lymphadenopathy, rash, jaundice, neurologic dysfunction, cytopenias, hyperferritinemia, hypertriglyceridemia, hypofibrinogenemia, elevated levels of the soluble IL-2 receptor (CD25), and diminished natural killer cell activity (Henter et al. 2007).

A recent literature review identified 69 reports of HPS after SOT: 60 in kidney (or kidney–pancreas), 5 in liver, 2 in heart, and 2 in lung transplant recipients. The mortality rate was 52 %. A precipitating viral infection was identified in approximately half of the cases (CMV in 20, EBV in 8, and other herpes viruses in 7 patients) (Diaz-Guzman et al. 2011). The largest published case series reported 17 cases of HPS after cadaveric renal transplant out of a total of 4,230 transplants for an overall incidence of 0.4 %. The median time to onset in this series was 52 days after transplant, and an infectious etiology was indentified in 14 of the patients (Karras et al. 2004). Management of HPS involves an aggressive search for and treatment of precipitating infection and supportive care.

Post-transplant Lymphoproliferative Disorder

PTLD refers to a family of predominantly EBV-driven, B-cell lymphoproliferative disorders that arise following hematopoietic stem cell or solid organ transplant. The disorder is caused by systemic immunosuppression, which impairs EBV-specific cytotoxic T-cell function, permitting expansion of B-cells latently infected with EBV. The EBV-infected B-cells that give rise to PTLD can originate in the recipient or the donor. PTLD following SOT is most commonly recipient derived. The clinical spectrum of PTLD after SOT ranges from an infectious mononucleosis-like polyclonal lymphoproliferation to aggressive non-Hodgkin's lymphoma (Smith 2010). The overall incidence at 10 years after SOT is approximately 1–2 %, with most cases occurring within the first year (Caillard et al. 2006; Andreone et al. 2003). Risk factors for the development of PTLD include the type of transplant (small bowel > heart

or lung > liver or kidney) (Cockfield 2001), the intensity of immunosuppression (Opelz and Henderson 1993), and the EBV serologic status of the donor and recipient (with the highest risk when an EBV-seronegative patient receives an organ from an EBV-seropositive donor) (Walker et al. 1995).

Recipient-derived PTLD is typically a multisystem disease characterized by constitutional symptoms, lymphadenopathy, and cytopenias if bone marrow involvement is present. Extranodal disease is also frequent, most commonly involving the gastrointestinal tract, lungs, skin, liver, and central nervous system. Donor-derived PTLD, in contrast, is often limited to the allograft tissue (Petit et al. 2002). The diagnosis of PTLD is suggested by an elevated EBV viral load in the peripheral blood (Stevens et al. 2001) and is confirmed by tissue biopsy. Management involves immediate reduction in immunosuppression to the minimal level required for preservation of the allograft. Rituximab monotherapy is added to treat disease that does not respond to reduced immunosuppression alone. Rituximab in combination with anthracycline-based chemotherapy is used for relapsed or refractory disease as well as in the initial treatment of cases associated with high-grade histology and a clinically aggressive course (Smith 2010).

Graft-Versus-Host Disease

Graft-versus-host disease (GVHD) is a rare and frequently fatal complication of SOT in which donor-derived T-cells in the transplanted organ engraft, proliferate, and attack tissues of donor origin. Transplantation of organs with a large quantity of lymphoid tissue (e.g., small bowel, liver) carries a greater risk of GVHD than organs with a lesser amount. Other reported risk factors include age greater than 65 and a greater degree of HLA match between donor and recipient (Kato et al. 2009).

The reported incidence rate of GVHD after orthotopic liver transplantation is 0.1–2 % (Smith et al. 2003) and less than 0.05 % after lung transplant (Worel et al. 2008). Clinical manifestations include fever, rash, diarrhea, and cytopenias and generally present within 2–8 weeks after transplant (Assi et al. 2007), though late occurrences have been reported (Pollack et al. 2005). The diagnosis is based on confirmation of lymphocyte chimerism in the peripheral blood, marrow, or affected tissues and tissue biopsy. Given the rarity of the disease, no evidence-based guidelines on treatment are available. Initial therapy is often with high-dose corticosteroids. Cases of successful treatment with antagonists of tumor necrosis factor-α have been reported (Piton et al. 2009; Thin et al. 2009). Consideration may be given to reducing immunosuppression to facilitate rejection of the allografted donor T-cells by the recipient immune system. Despite these efforts, mortality remains well in excess of 50 % in most reports (Pavenski et al. 2008).

Post-transplant Immune Thrombocytopenia

ITP, either alone or in combination with autoimmune hemolytic anemia (Evans syndrome), has been rarely reported as a complication of SOT, possibly as a result of a central tolerance defect induced by systemic immunosuppression (Cines et al. 2009). In a pediatric series of 158 liver transplants, the incidence of ITP was 1.9 % (Miloh et al. 2011). Substantial clinical heterogeneity has been observed in reported cases. ITP may arise in the immediate post-transplant period or months later and may be self-limited and responsive to conventional ITP therapies or assume a chronic, refractory course (Cines et al. 2009). In one report, ITP was transferred from a donor to a recipient after orthotopic liver transplant (Friend et al. 1990). ITP is a diagnosis of exclusion. Treatment is discussed elsewhere (see Chap. 7).

Diagnostic Approach

Determination of the etiology of thrombocytopenia in SOT patients is often challenging. We consider the time of onset of thrombocytopenia in relation to transplant in developing our differential

Table 8.5 An approach to the diagnosis of thrombocytopenia after SOT. Our initial differential diagnosis is formulated, in part, on the time of onset of thrombocytopenia after SOT. Specific laboratory and pathologic testing, when available, is used to confirm the diagnosis

Etiology	Typical time of onset after SOT	Diagnostic testing
TMA	Median 7 days	Microangiopathic changes on smear
GVHD	2–8 weeks	Lymphocyte chimerism
		Tissue biopsy
Viral infection	1–6 months	Varies depending on virus
Infection-induced HPS	1–6 months	See consensus criteria (Henter et al. 2007)
		Hemophagocytosis on biopsy
PTLD	Within 1 year	EBV PCR Tissue biopsy
Drug induced	Any time, often within several weeks of starting a new medication	–
ITP	Any time	–

SOT solid organ transplant, *TMA* thrombotic microangiopathy, *GVHD* graft-versus-host disease, *HPS* hemophagocytic syndrome, *PTLD* post-transplant lymphoproliferative disorder, *EBV* Epstein–Barr virus, *PCR* polymerase chain reaction, *ITP* immune thrombocytopenia

diagnosis and request definitive testing, when available, to confirm the diagnosis (Table 8.5). TMA generally occurs in the early post-transplant period with a median onset at day 7 (Said et al. 2010). Identification of microangiopathic changes on the peripheral blood smear is critical for verifying the diagnosis. GVHD generally arises 2–8 weeks after transplantation and is confirmed with lymphocyte chimerism studies and biopsy of affected tissue. Viral infections may occur at any time during the post-transplant course but are most common between 1 and 6 months when patients suffer the greatest impact of immunosuppression. Confirmatory testing for CMV, the most common infection in this setting, involves quantitative PCR or an antigen assay. Infection-induced HPS tends to occur in the same time frame as the infections that drive it. Diagnostic criteria for this condition have been published (Henter et al. 2007), among them evidence of hemophagocytosis on biopsy of the bone marrow or the lymphoid tissue. Most cases of PTLD occur in the first year. The diagnosis is suggested by EBV quantitative PCR and confirmed by biopsy of affected tissue. Drug-induced thrombocytopenia may occur at any time after transplant but is most likely to arise within several weeks of initiation of a new medication. ITP may occur at any time after SOT and remains a diagnosis of exclusion from other causes.

Quantitative PCR is consistent with CMV viremia. Bone marrow biopsy demonstrates hypocellularity (20 %) but otherwise normal trilineage hematopoiesis. Lymphocyte chimerism studies are negative for the presence of donor lymphocytes. CMV disease is suspected, and ganciclovir is initiated with resolution of the patient's rash and fever and rapid improvement in blood counts.

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Thrombocytosis

9

Stephan Lindsey and Ramon V. Tiu

Reactive Causes of Thrombocytosis (Secondary Thrombocytosis)

Clinical Vignette 1

A 63-year-old woman is admitted in the hospital for fever (T = 104 °F), cough, and pleuritic chest pain that started 5 days prior. Vital signs on admission showed persistent fever, elevated respiratory rate (RR = 25), and decreased O_2 saturation by pulse oximetry (O_2 sat = 89 %). On physical examination, the patient is in respiratory distress and lungs have decreased breath sounds and crackles on the left lower lung; the rest of the examination is unremarkable. Complete blood count (CBC) shows a hemoglobin of 12.7 g/dl, mean corpuscular volume of 96 fl, and leukocyte count of 18,000/μL, with an absolute neutrophil count of 16,000/μL and platelet count of 625,000/μL. A chest X-ray showed left lower lobe pneumonia.

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Infection

General Features

Infection is a common cause of reactive thrombocytosis in both children and adults (Table 9.1). It accounts for 22–31 % of cases of thrombocytosis in several large studies (Santhosh-Kumar et al. 1991; Griesshammer et al. 1999; Buss et al. 1994; Dame and Sutor 2005), and it is true even for patients with extreme thrombocytosis (31 %) (Buss et al. 1994). In children, one-third to two-thirds of cases of reactive thrombocytosis are due to bacterial or viral infections involving the respiratory, gastrointestinal, or urinary tract (Vannucchi and Barbui 2007). The most common infection associated with thrombocytosis in both adults and children is pneumonia (31 %) (Griesshammer et al. 1999; Vlacha and Feketea 2006), but thrombocytosis can also be seen in patients with other severe infections such as gastrointestinal (11 %), soft tissue (17 %), and hepatobiliary infections (7 %) (Griesshammer et al. 1999).

The main cause of thrombocytosis in cases of severe infections may be a rebound phenomenon after initial thrombocytopenia due to rapid consumption of platelets. The mechanism by which reactive thrombocytosis is triggered during severe infections typically involves an increased release of numerous cytokines, especially thrombopoietin (TPO), the primary cytokine for platelet production and maturation, and interleukin (IL)-6. While serum or plasma levels of these cytokines do not seem to correlate with the degree of thrombocytosis, increased TPO and IL-6 levels

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Table 9.1 Causes of thrombocytosis

Primary	Secondary
Myelodysplastic syndromes (MDS)	*Nonmalignant hematological conditions*
5q- syndrome	Acute hemolytic anemia
Myeloproliferative neoplasms (MPN)	Iron-deficiency anemia
Essential thrombocythemia (ET)	Acute bleeding
Polycythemia vera (PV)	Posttreatment of vitamin B_{12} deficiency
Chronic myelogenous leukemia (CML)	*Malignant hematological conditions*
Primary myelofibrosis (PMF)	Metastatic cancer
Post-ET myelofibrosis	Lymphoma
Post-PV myelofibrosis	Posttreatment with myelosuppressive drugs
MDS/MPN overlap	*Acute and/or chronic inflammatory conditions*
Refractory anemia with ring sideroblasts associated with marked thrombocytosis (RARS-T)	Infections
	Connective tissue disorders (vasculitis)
	Inflammatory bowel disease (IBD)
	Autoimmune disease (celiac disease)
	Post-splenectomy or functional asplenia
	POEMS syndrome (osteosclerosis myeloma)
	Tissue damage
	Myocardial infarction
	Severe trauma
	Acute pancreatitis
	Thermal burns

are believed to spur megakaryocytic differentiation and result in increased platelet release. Any infectious agents can cause thrombocytosis although bacterial infections are usually the most predominant cause.

Other Causes of Reactive Thrombocytosis

Malignancy

General Features

Thrombocytosis is observed in 10–57 % of patients with cancer (Sierko and Wojtukiewicz 2004), with the most likely cancers to cause thrombocytosis are lung, breast, lymphoma, ovarian, and gastrointestinal cancers (Bambace and Holmes 2011). For the majority of malignancies, the extent of platelet count elevation is inversely correlated with survival, making thrombocytosis a marker of poor prognosis (Ikeda et al. 2002; Lopes et al. 1994; Monreal et al. 1998; Gucer et al. 1998).

The specific pathogenesis of thrombocytosis in malignancy has not yet been identified, but tumor cells are known to secrete cytokines such as IL-1, GM-CSF, G-CSF, and IL-6, which stimulate megakaryocytic differentiation and platelet generation through a thrombopoietin-dependent mechanism (Gastl et al. 1993; Suzuki et al. 1992; Kaser et al. 2001; Estrov et al. 1995). Additional inflammatory cytokines are then released by megakaryocytes, resulting in an effective amplification of the original cytokine signals (Wickenhauser et al. 1995). Further amplification of megakaryopoietic differentiation signals occurs when megakaryocytes and platelets release vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (b-FGF), two factors known to impact megakaryocytic maturation via an autocrine loop (Mohle et al. 1997; Avraham et al. 1994; Casella et al. 2003).

Connective Tissue Disease/Other Autoimmune Diseases

General Features

The incidence of thrombocytosis resulting from autoimmune diseases varies greatly between the diseases causing the thrombocytosis, with estimates ranging from less than one newly diagnosed case of systemic sclerosis to more than 20 cases of adult-onset rheumatoid arthritis per 100,000 person-years (Cooper and Stroehla 2003). Prevalence rates range from less than 5 per 100,000 (e.g., chronic active hepatitis, uveitis) to more than 500 per 100,000 (Graves disease, rheumatoid arthritis, thyroiditis), although the reported prevalence and incidence of connective tissue disorders are quite variable, depending on differences in study methodology. The most common connective tissue disease associated with thrombocytosis is systemic lupus erythematosus (SLE). The prevalence of SLE is estimated between 15

and 50 per 100,000 individuals, with a female:male ratio of 6–10:1 in the age group between 15 and 40 years (Gaubitz 2006). Thrombocytosis occurs in 20–50 % of patients with severe rheumatoid arthritis but also occurs in patients with inflammatory bowel disease and other autoimmune diseases (Ehrenfeld et al. 1977).

The specific causes of most connective tissue diseases are not known, but many connective tissue diseases feature chronic inflammation, usually due to abnormal immune system activity and autoimmunity. Connective tissue diseases act similarly to infections, inflammation, and autoimmune diseases in that they result in an accumulation and release of IL-6. The elevated levels of IL-6 in these diseases result in accelerated megakaryocytic differentiation, leading to elevated platelet counts and thrombocytosis. However, occasionally patients with SLE develop thrombocytosis as a result of autosplenectomy, a progressive shrinking of the spleen that occurs as healthy spleen tissue is replaced by nonfunctional fibrous tissue. In these cases, thrombocytosis results from an inability of the spleen to remove older circulating platelets.

As with thrombocytosis resulting from other forms of inflammation, there is a direct correlation between increased IL-6 concentrations in autoimmune diseases and platelet counts (de Benedetti et al. 1991). In active inflammatory bowel diseases, TPO concentrations are also elevated but do not correlate with increased platelet counts. Because changes in TPO concentrations generally precede thrombocytosis, a model has been proposed whereby increased TPO and IL-6 result in thrombocytosis in inflammatory bowel disease and other autoimmune diseases (Papa et al. 2003).

Post-surgery

General Features

Thrombocytosis can occur after major surgeries, with a maximal rise in platelet counts (often more than doubled) from 7 to 20 days post surgery (Ruggeri et al. 2008). The mechanism for postoperative thrombocytosis remains unclear, but increased circulating TPO levels are believed to play a role (Folman et al. 2001). One possible mechanism for this phenomena is that surgical procedures may activate platelets, leading to TPO release and a corresponding increase in circulating TPO levels. This rise in circulating TPO levels could then in turn stimulate proliferation and differentiation of megakaryocytic cells, eventually resulting in the release of platelets in the circulation (Kaushansky et al. 1995; Cramer et al. 1997).

Diagnosis

The diagnosis of reactive thrombocytosis resulting from infections, cancer, and connective tissue/autoimmune disease is made by identification of the specific disease etiology in the absence of alternative causes of thrombocytosis. In terms of patients who underwent surgery, postsurgical thrombocytosis can be diagnosed if there are no other obvious causes of thrombocytosis. A bone marrow biopsy is not always indicated.

Management/Prognosis

Reversal of the primary infection, eradication or control of the underlying cancer, and treatment of connective tissue/autoimmune disease usually result in resolution of thrombocytosis. For postsurgical cases, platelet counts usually decrease several days after surgery. Persistence of thrombocytosis when the underlying putative cause has resolved should warrant additional work-up for alternative causes of thrombocytosis. When appropriate, low-dose aspirin may be started. Sometimes, when the platelet counts are high and vasomotor or thrombotic symptoms become apparent in a patient with no clonal source of thrombocytosis, cytoreductive therapy may be indicated. However, data supporting definitive recommendations on the best cytoreductive therapy, duration of treatment, and interval of treatment are lacking. The prognosis of patients with thrombocytosis in the setting of infection, cancer, and connective tissue/autoimmune diseases is primarily driven by the underlying disease.

Clinical Vignette 1 (Continued)
The patient is treated with ceftriaxone IV and azithromycin. The patient's fever defervesced in 3 days, and the repeat platelet count on the fifth day of admission was 225,000/μL.

Clinical Vignette 2
A 65-year-old man is evaluated for pallor and melena. On physical examination, the patient looked pale with no other obvious findings. Complete blood count shows a hemoglobin of 9.8 g/dl, MCV of 74 fl, and platelet count of 522,000/μL. Fecal occult was positive in three separate samples. Iron studies showed a serum Fe of 10 μg/dl, total iron binding capacity (TIBC) of 670 μg/dl, transferrin saturation of 2 %, and serum ferritin of 5 ng/ml.

Iron-Deficiency Anemia

General Features

Iron-deficiency anemia is a common anemia caused by insufficient dietary intake and absorption of iron and/or iron loss from gastrointestinal or genitourinary (menstrual) bleeding. Iron deficiency causes approximately half of all anemia cases worldwide and affects women more often than men. World estimates of iron deficiency occurrence are somewhat vague, but the true number probably exceeds one billion persons (Stoltzfus 2001). The estimated prevalence of iron deficiency was greatest among toddlers aged 1–2 years (7 %) and adolescent and adult females aged 12–49 years (9–16 %) (Cogswell et al. 2009). The incidence of iron-deficiency anemia drops to 6–9 % in postmenopausal women. The prevalence of iron deficiency is two times higher among non-Hispanic black and Mexican-American females (19–22 %) than among non-Hispanic white females (10 %). Excluding persons aged ≥3 years with elevated C-reactive protein levels (>1 mg/dL) from the analysis did not change prevalence estimates.

Iron-deficiency anemia is the most common form of anemia and develops over time if the body does not have enough iron to manufacture red blood cells. Either diminished absorbable dietary iron or excessive loss of body iron can cause iron deficiency. Without enough iron, the body uses up all the iron it has stored in the liver, bone marrow, and other organs. Once stored iron is depleted, the body is able to make very few red blood cells. The red blood cells the body is able to make are abnormal and do not have a normal hemoglobin-carrying capacity compared to normal red blood cells. The most common cause of body iron loss is hemorrhage, including excessive menstrual flow in premenopausal women. However, iron loss can also occur with hemoglobinuria from intravascular hemolysis. Malabsorption of iron is relatively uncommon in the absence of small bowel disease such as celiac disease or regional enteritis or previous GI surgery.

The mechanism by which iron-deficiency anemia leads to thrombocytosis remains elusive, but a feedback mechanism involving erythropoietin (EPO) and thrombopoietin may be involved. Reduced levels of EPO, the predominant cytokine responsible for erythrocytic proliferation and differentiation, may trigger megakaryocytic differentiation and eventual platelet biogenesis/release.

Diagnosis

The diagnosis of iron-deficiency anemia is made by ordering iron studies. Thrombocytosis from iron-deficiency anemia resolves after adequate iron supplementation. If thrombocytosis persists, work-up to exclude other causes should be conducted.

Treatment/Prognosis

The treatment of thrombocytosis in the setting of iron-deficiency anemia is by supplementation of iron either given intravenously or orally and identification and management of the underlying cause of the iron-deficiency anemia. The thrombocytosis in patients with iron-deficiency anemia

does not generally affect the prognosis of patients. However, the most common cause of iron-deficiency anemia in adults is gastrointestinal bleeding. In the elderly, colorectal cancers are typically the cause, and so a careful visualization and biopsy of the GI tract are necessary (Alleyne et al. 2008).

Clinical Vignette 2 (Continued)
The patient was diagnosed with iron-deficiency anemia and underwent a gastrointestinal work-up. An upper gastrointestinal endoscopy was performed and showed four bleeding ulcers in the antrum of his stomach. All bleeding ulcers were cauterized, and the patient was started on a proton pump inhibitor. Test for *H. pylori* was negative. He was started on intravenous iron supplementation with ferric gluconate 125 mg IV once a week for 8 weeks. He had no further bleeding episodes, and his repeat iron studies showed a serum Fe of 42 μg/dl, TIBC of 270 μg/dl, transferrin saturation of 35 %, and serum ferritin of 335 ng/ml. Repeat CBC showed a Hgb of 13 g/dl, an MCV of 85 fl, and a platelet count of 338,000/μL.

Clinical Vignette 3
A 55-year-old man had a vehicular accident and had a splenic rupture. Emergency splenectomy was performed. He has no cardiovascular risk factors like diabetes mellitus or hyperlipidemia. He is a nonsmoker. On physical examination, the patient was in distress from pain in his left upper quadrant. CBC shows a hemoglobin of 13.2 g/dl and platelet count of 200,000/μL. Two days immediately post-op, a repeat CBC showed a hemoglobin of 12.1 g/dl and platelet count of 950,000/μL.

Post-splenectomy

General Features

Open or laparoscopic splenectomy are surgical approaches sometimes performed for diagnostic and therapeutic purposes. Both procedures can sometimes be complicated by bleeding, infections, thrombosis, and thrombocytosis. Thrombocytosis that occurs post splenectomy may be a consequence of reactive thrombocytosis of decreased clearance of old platelets. The incidence is about 75–82 % (Khan et al. 2009). The etiology of thrombosis occurring post splenectomy has been postulated to be from multiple causes including pneumoperitoneum (12 mmHg) that reduces the venous flow and causes venous pooling in the legs. Prophylactic splenectomy can result in increased platelet adhesiveness if associated with persistent thrombocytosis. Other factors include higher D-dimer, prothrombin fragment 1 and 2, lower protein C and S, and increased fibrinogen and thrombin activatable fibrinolysis (Hirsh et al. 1966; Tripatara et al. 2007). The incidence of thrombosis post splenectomy may vary depending on the indication with high frequency in patients splenectomized for an MPN.

Diagnosis

Thrombocytosis is frequently observed post splenectomy, and platelet counts can range anywhere from 600,000 to 800,000/μl although extreme cases of thrombocytosis have been observed. The peak platelet levels usually occur 7–20 days post surgery (Greer et al. 1981).

Treatment/Prognosis

Prophylactic post-surgery anticoagulation is important if there are no contraindications. Low-dose aspirin with or without cytoreductive therapy may be started in some patients who are at high risk or are developing thrombotic symptoms. Cytoreductive therapies that can be used include hydroxyurea, anagrelide, or interferon, similar to what is used in patients with MPN. However, guidelines based on definitive data supporting this recommendation are not available.

Clinical Vignette 3 (Continued)
Seven days post surgery, his platelet count was stable at 950,000/μL with no thrombotic events. He was started on aspirin 81 mg PO once daily and was discharged out of the hospital.

Clinical Vignette 4
A 60-year-old man has a long-standing history of immune-mediated thrombocytopenia. He previously received steroids and intravenous immunoglobulin but with no response. He is currently being treated with romiplostim 5 mcg/kg/week which is started 3 weeks ago, and he missed the weekly blood tests scheduled since starting the therapy because of transportation problems. He is scheduled to see his hematologist in another week but had to go to a local ER because of a cut on his left arm sustained during carpentry work. His physical examination showed a 12 cm laceration on his left arm. Complete blood count shows a white blood cell count of 15,000, hemoglobin of 14.1 g/dl, and platelet count of 780,000/μL. The ER physician called a hematology consult to assess the cause of the thrombocytosis.

Medication-Induced Thrombocytosis

Thrombopoietin Agonists

General Features

Thrombopoietin agonists such as romiplostim and eltrombopag are often used to treat cases of thrombocytopenia, especially when resulting from immune thrombocytopenic purpura (ITP), a hematologic disease with a prevalence of about 5 in 100,000 (Ikeda and Miyakawa 2009). Acute ITP is more common in children than adults and displays no racial preference, while chronic ITP is more common in adult women between 20 and 40 years old.

Platelets and megakaryocytes physiologically respond and regulate plasma thrombopoietin levels by binding TPO to the *c-mpl* cell surface receptor. When platelet counts are increased, more plasma TPO is bound to the cells, resulting in reduced levels of plasma TPO. Conversely, when platelet counts are low, increased levels of plasma TPO are available to stimulate megakaryopoiesis within the bone marrow. By regulating plasma TPO levels, platelet formation is maintained at a steady state under normal circumstances and plasma TPO levels are inversely correlated to platelet counts and megakaryocytic differentiation (Hou et al. 1998). When therapeutic TPO agonists are given to patients, this physiologic feedback system is lost, as plasma TPO levels remain high no matter what the platelet counts of the patient are.

Diagnosis

The diagnosis of a TPO agonist-induced thrombocytosis should be suspected in any patient receiving treatment with romiplostim, eltrombopag, or other TPO agonists.

Treatment/Prognosis

TPO agonist-induced thrombocytosis is managed by reduction of the original dose or by temporarily withholding therapy until the thrombocytosis has resolved while carefully monitoring platelet counts so that reciprocal severe symptomatic thrombocytopenia does not occur. Table 9.2 describes the starting dose and dose adjustment recommendations for romiplostim and eltrombopag, depending on platelet counts. The concern for elevated platelet counts in the setting of TPO agonist-induced thrombocytosis is the risk of developing thrombotic events. In a study evaluating the number of thrombotic events after long-term use of romiplostim in patients with chronic ITP, the exposure-adjusted incidence of thrombosis is 0.1 per 100 patient-weeks in the Phase III study and 0.08 per 100 patient weeks in the extension study (Gernsheimer et al. 2010). If platelet counts remain persistently elevated several weeks after discontinuation of TPO agonist, then alternative causes of thrombocytosis should

Table 9.2 Dosing recommendations for romiplostim and eltrombopag

	Romiplostim	Eltrombopag	Eltrombopag
Indication	ITP	ITP	Hepatitis C-induced thrombocytopenia
Starting dose	1 mcg/kg of actual body weight	50 mg (25 mg for patients of East Asian ethnicity)	25 mg
Route of administration	SC	PO	PO
Dosing schedule	Weekly	Daily	Daily
Maximum dose	10 mcg/kg	75 mg	100 mg
Dose adjustment	*Platelet count <50,000/μL* Increase weekly dose by 1 mcg/kg	*Platelet count <50,000/μl (≥2 weeks after treatment initiation or a dose increase)* Increase daily dose by 25 mg[a]	*Platelet count <50,000/μl (≥2 weeks after treatment initiation)* Increase daily dose by 25 mg every 2 weeks
	Platelet count >200,000/μL (2 consecutive weeks) Reduce weekly dose by 1 mcg/kg	*Platelet count ≥200,000/μl and ≤400,000/μl (at any time)* Decrease daily dose by 25 mg and reevaluate in 2 weeks	*Platelet count ≥200,000/μl and ≤400,000/μl (at any time)* Decrease daily dose by 25 mg and reevaluate in 2 weeks
	Platelet counts >400,000/μL: Dose should be held and platelet counts monitored once weekly, and then once the platelet count is <200,000/μL then the weekly dose should be re-started at a dose 1 mcg/kg less than the prior dose	*Platelet count >400,000/μl*: Hold dose; assess platelet count twice weekly; when platelet count is <150,000/*μl*, resume with the daily dose reduced by 25 mg[b]	*Platelet count >400,000/μl*: Hold dose; assess platelet count twice weekly; when platelet count is <150,000/*μl*, resume with the daily dose reduced by 25 mg[b]
		Platelet count >400,000/mm³ after 2 weeks at the lowest dose Discontinue therapy	*Platelet count >400,000/mm³ after 2 weeks at the lowest dose* Discontinue therapy

[a]If the patient is taking 12.5 mg daily, increase dose to 25 mg daily before increasing the dose by 25 mg daily
[b]If taking 25 mg daily, resume with 12.5 mg daily

be considered and evaluated. The prognosis of patients with TPO agonist-induced thrombocytosis is primarily affected by the development of thrombotic events and by the severity and natural history of the underlying disease being treated with the TPO agonist.

Clinical Vignette 4 (Continued)

The patient was admitted in the hospital for work-up. The romiplostim dose was held, and platelet counts were checked daily while the patient was in the hospital. By day 7 of hospitalization, platelet counts were 380,000/μL. No thrombotic events occurred during the hospitalization. He was seen 1 week later by his local hematologist who started him on 4 mcg/kg SC once a week of romiplostim. The repeat CBC the following week showed a platelet count of 371,000/μL. Patient has no bleeding episodes.

Other Medications

Vincristine-Induced Thrombocytosis

General Features

Vinca alkaloids like vincristine, a mitotic spindle inhibitor, is a commonly used therapeutic agent in the management of several hematologic and solid malignancies. It is part of the backbone regimen of R-CHOP for patients with diffuse large B cell lymphomas and of CODOX-M/IVAC for Burkitt's lymphomas. The clinical observation that vincristine causes thrombocytosis was made back in the 1960s (Carbone et al. 1963; Robertson and McCarthy 1969). It is one of the therapeutic options for patients with refractory ITP. When low doses of vincristine are administered in mice, thrombocytosis without preceding thrombocytopenia is observed. The mechanisms leading to thrombocytosis after vincristine administration include compensatory changes in megakaryocytes due to suppression of megakaryocytes

and their progenitors (Harris and Penington 1984) and alterations in the function role of circulating platelets in regulating platelet production (Jackson and Edwards 1977).

Low-Molecular-Weight Heparin (Enoxaparin and Others)

General Features

Anticoagulation using heparin-based therapy is commonly used for both prophylactic and therapeutic management of patients who are at risk or have developed arterial and venous thromboembolic events. One of the most commonly used anticoagulants is enoxaparin, a low-molecular-weight heparin (LMWH), which works primarily by enhancing the inhibitory properties of clotting proteases by antithrombin which leads to inhibition of factor Xa and blocking normal hemostasis. It differs from standard heparin in that it has a lower molecular weight (4,500 Da), has a higher ratio of anti-factor Xa and anti-factor IIa activity compared to standard unfractionated heparin, and does not require regular monitoring. Although sometimes implicated as a potential cause of heparin-induced thrombocytopenia, enoxaparin can also lead to thrombocytosis (Rizzieri et al. 1996; Williams 1997). The exact mechanism for the thrombocytosis is not known but is believed to be due to a reactive process. This phenomenon is not exclusive to enoxaparin as other LMWH such as nadroparin, dalteparin, and reviparin have also been noted to cause thrombocytosis (Liautard et al. 2002).

All-Trans Retinoic Acid

General Features

All-trans retinoic acid (ATRA) is one of the most important treatments of patients with acute promyelocytic leukemia (APL). ATRA induces the in vitro and in vivo differentiation of APL cells and contributes to the cure of patients with APL when given in conjunction with chemotherapy. The most frequently associated adverse event during ATRA therapy is the development of differentiation syndrome. Another less common and infrequently recognized adverse event is the development of thrombocytosis which in one series reported a frequency of 23 %. It is suggested that the increase in IL-6 induced by ATRA may be the cause of the elevated platelet counts (Losada et al. 1996).

Diagnosis

Other medications that may lead to thrombocytosis include epinephrine, glucocorticoids, and interleukin-1B. The diagnosis of a medication-induced thrombocytosis should be suspected in a patient with high platelet counts with no other alternative causes of thrombocytosis and currently receiving any of the abovementioned therapies.

Management/Prognosis

Discontinuation of the thrombocytosis-inducing drug usually leads to normalization of the platelet counts or a return of platelet counts to baseline. In patients treated with LMWH, none of the patients developed thrombotic events (Liautard et al. 2002). In general, no specific therapies are indicated for medication-induced thrombocytosis unless symptoms develop. If thrombocytosis persists after drug discontinuation, then evaluation for other causes of thrombocytosis may be necessary.

Clonal Causes of Thrombocytosis (Primary Thrombocytosis)

Clinical Vignette 5

A 44-year-old man was admitted in the hospital for fatigue, palpitations, drenching night sweats, and shortness of breath. He had unintentional weight loss of 20 lbs in the past 2–3 months. On physical examination, the spleen was palpable 15 cm below the left subcostal margin; the rest of the examination was unremarkable. CBC showed a hemoglobin of 7.3 g/dl, mean ()()

(continued)

corpuscular volume of 105 fl, leukocyte count of 40,000/μL with an absolute neutrophil count of 32,000/μL with occasional metamyelocytes, absolute eosinophil count of 3,000/μL, and platelet count of 585,000/μL. He had no fever and no obvious evidence of active infection.

Myeloproliferative Neoplasms

Chronic Myeloid Leukemia, *BCR–ABL1* Positive

General Features

Chronic myeloid leukemia (CML) represents about 15–20 % of all cases of adult leukemia in Western populations (Zhang and Rowley 2011). CML occurs in all age groups but most commonly in the middle aged and elderly. Its annual incidence is 1–2 per 100,000 people, with a slightly higher male predilection. The only well-described risk factor for CML is exposure to ionizing radiation (Moloney 1987). It is a clonal neoplasm of hematopoietic progenitor cells and involves the myeloid, monocytic, erythroid, megakaryocytic, B-lymphoid, and occasionally T-lymphoid lineages (Faderl et al. 1999). Central to its pathogenesis is the BCR–ABL fusion protein that generally results from the chromosomal rearrangement t(9;22)(q34;q11). Chronic-phase CML resembles a benign expansion of myelopoiesis where myeloid progenitor cells expand at various stages of maturation, are released prematurely into the peripheral blood, and relocate to extramedullary locations. Thrombocytosis of >700,000/μl as an initial finding can be found in 15–35 % of CML patients, while extreme thrombocytosis is less common. Patients with CML with very high platelet counts may be at greater risk of thrombotic or hemorrhagic complications.

Diagnosis

The diagnosis of CML is usually suspected in a patient with elevated white blood cell count with a left shift, constitutional symptoms, and enlarged spleen. However, 20–40 % of patients may be asymptomatic at presentation and may just present with persistently elevated WBC. In the peripheral blood, neutrophilia and the presence of immature myeloid cells are important features of CML with >50 % of patients presenting with a WBC count of >100,000/μl. Blasts usually account for <2 % of the total WBC in most cases. Other commonly observed peripheral blood changes are absolute basophilia and eosinophilia. Anemia may be present in up to half of patients. The diagnosis is based on the identification of the Philadelphia chromosome or the presence of the BCR–ABL fusion gene or transcript either by fluorescence in situ hybridization (FISH) or by reverse transcription-polymerase chain reaction (RT-PCR) methods.

Treatment/Prognosis

The current frontline treatment for patients with newly diagnosed chronic phase is a tyrosine kinase inhibitor (TKI). Various TKI are now FDA approved for the treatment of CML including imatinib, dasatinib, nilotinib, bosutinib, and ponatinib. For newly diagnosed chronic-phase CML, imatinib, dasatinib, and nilotinib are FDA approved for this indication. Bosutinib, dasatinib, and ponatinib are FDA approved for the second-line treatment of all phases of CML. Nilotinib is FDA approved for the second-line treatment of chronic- and accelerated-phase CML. Another agent now FDA approved for the treatment of CML is a protein translation inhibitor, omacetaxine mepesuccinate (Jabbour and Kantarjian 2012). Thrombocytosis usually resolves after successful therapy of CML. Allogeneic stem cell transplantation (SCT) can be potentially curative to some patients. Prognosis of patients with CML in the era of TKI has improved. A single-institution study showed the trend in the improvement of overall survival over time pre-TKI and during the TKI era. In chronic-phase CML, the 8-year survival was 87 % since the year 2001 compared to ≤15 % before the year 1983 and 42–65 % from the year 1983 to 2000 (Kantarjian et al. 2012).

Polycythemia Vera

General Features

The annual incidence of polycythemia vera (PV) is about 2 per 100,000 inhabitants. Polycythemia vera occurs in all age groups, although the incidence increases with age. The median age at diagnosis is between 60 and 70 years of age (Johansson 2006; Tefferi 2012), although polycythemia vera has also been reported in children (Passamonti et al. 2003).

Polycythemia vera is caused by the neoplastic proliferation and maturation of erythroid, megakaryocytic, and granulocytic cells. In contrast to secondary polycythemias, polycythemia vera is associated with a low serum level of the hormone EPO. The majority of PV patients (>95 %) have a point mutation in the *JAK2* gene (Levine et al. 2005). This leads to constitutive activation of the JAK–STAT pathway. In thrombocytosis, the point mutation (*JAK2*V617F) makes hematopoietic progenitor cells in PV patients hypersensitive to TPO, leading to proliferation of megakaryocytes and their differentiation into platelets.

Diagnosis

In the setting of thrombocytosis, PV is suspected when other clinicopathologic features of the disease are also present. The vast majority of PV patients are symptomatic at presentation. Symptoms described include pruritus, fatigue, dizziness, plethora, epigastric pain, weight loss, erythromelalgia, dyspnea, paresthesias, and headaches. Some may have symptomatic splenomegaly as well. The CBC of the patient will most of the time reveal an elevated hemoglobin/hematocrit level. However, this may not always be true for some patients with concomitant iron-deficiency anemia or volume expansion from another concomitant disease. The diagnosis of PV can be made by fulfilling the criteria set by the 2008 WHO classification. Although thrombocytosis can occur in PV patients, it is not part of the criteria to diagnose PV (Table 9.3). A bone marrow biopsy is not always indicated but may be useful in unusual presentations and in excluding other diseases that may mimic PV.

Treatment/Prognosis

Qualitative and quantitative abnormalities in platelets can be present in PV patients. Qualitatively, abnormally low levels of adenine and serotonin and defects in in vitro aggregation responses to epinephrine and/or collagen can be seen in PV. Quantitatively, thrombocytosis and even extreme thrombocytosis are observed. When platelet counts are elevated and clinical bleeding is occurring due to the qualitative platelet defect, platelet transfusions (of normal platelets) can help. In cases of extreme thrombocytosis, acquired type 2 von Willebrand disease (vWD) may occur which can lead to clinical bleeding. This results from increased clearance of large vWF multimers by the elevated platelet counts. Testing for ristocetin cofactor activity may help diagnose this problem. Plateletpheresis and cytoreductive agents like hydroxyurea, anagrelide, pegylated interferon, and busulfan can help reduce the platelet count and restore the normal levels of large vWF multimers. Generally, patients with PV should be managed with phlebotomy, to keep the Hct at <45 %, and low-dose aspirin (Marchioli et al. 2013). The decision to give cytoreductive therapy is still primarily driven by the risk of thrombotic events. Generally, high-risk PV patients (>60 years old and/or had a prior history of blood clots) need cytoreductive therapy together with phlebotomy and low-dose aspirin, while low-risk PV patients (<60 years old and/or no history of blood clots) can be managed with just phlebotomy and low-dose aspirin (Reikvam and Tiu 2012; Barbui et al. 2012). Several JAK inhibitors are also undergoing clinical trials in patients with PV. Patients diagnosed at a median age of 60–65 years old appropriately treated with phlebotomy and low-dose aspirin with or without cytoreductive therapy have long-term outcomes with some having near-normal life-spans. However, the median survival of PV patients diagnosed at <40 years old is shorter than the life expectancy for a healthy person of similar age, even with active management. The only known potential curative option for PV is an allogeneic HCT. Data from the Fred Hutchinson Cancer Research

Table 9.3 Diagnostic criteria for MDS, MDS/MPN, and Philadelphia chromosome negative MPN by 2008 WHO criteria

Disease subtype	Criteria
MDS	
MDS MDS associated with isolated del(5q)	(1) Anemia (2) Normal/high platelet counts (3) BM blasts <5 %, and PB blasts <1 % (4) Normal to increased megakaryocytes with hypolobulated nuclei (5) Absence of Auer rods (6) Presence of an isolated del(5q) chromosomal abnormality
MDS/MPN	
MDS/MPN-U (RARS-T)	(1) Platelet count ≥450×10^9/L (2) Presence of ring sideroblasts (RS) in the BM (≥15 % RS) (3) Presence of atypical megakaryocytes (4) No blasts in peripheral blood (5) <5 % blasts in the bone marrow (6) Anemia (7) Ineffective erythroid proliferation with megaloblastoid features
PH-negative MPN	
(PMF)[a]	Major criteria (1) Presence of megakaryocyte proliferation and atypia with reticulin or collagen fibrosis or if fibrosis is absent then the megakaryocyte changes must have increased BM cellularity with granulocytic proliferation (2) Positive for JAK2V617F or MPL W515K/L or if clonal marker is absent no other secondary causes of fibrosis (3) Not meeting the criteria for another myeloid neoplasm Minor criteria (1) Increased LDH (2) Leukoerythroblastosis (3) Splenomegaly (4) Anemia
(PV)[b]	Major criteria (1) Increased Hgb (M:>18.5 g/dL; F:>16.5 g/dL) or other evidence of increased red cell volume (2) Positive *for JAK2*V617F or another functional mutation Minor criteria (1) Hypercellular BM with increased erythroid, granulocytic, and megakaryocytic proliferation (2) Spontaneous cellular growth in vitro (3) Serum EPO level below the reference range for normal
(ET)[c]	(1) Platelet count ≥450×10^9/L (2) Presence of increased enlarged mature megakaryocytes (3) Positive for *JAK2*V617F or other clonal markers or in the absence of a clonal marker, no evidence of reactive thrombocytosis (4) Not meeting the criteria for another type of myeloid neoplasm

Diagnostic criteria are given at disease presentation. *BM* bone marrow, *PB* peripheral blood, *M* male, *F* female, *LDH* lactate dehydrogenase, *Hgb* hemoglobin, *EPO* erythropoietin, *MDS* myelodysplastic syndrome, *MDS/MPN* myelodysplastic syndrome/myeloproliferative neoplasms, *RARS-T* refractory anemia with ring sideroblasts associated with thrombocytosis, *CML* chronic myeloid leukemia, *PMF* primary myelofibrosis, *PV* polycythemia vera, *ET* essential thrombocytosis

[a]Must fulfill all three major and two minor criteria

[b]Must fulfill two major and one minor criteria or the first major and second minor criteria

[c]Must fulfill all four criteria

Center and Center for International Blood and Marrow Transplant Research Centers showed that allogeneic HCT in PV results on a 5-year survival/progression-free survival of 47 and 48 % (Ballen et al. 2012).

Essential Thrombocythemia

General Features

Essential thrombocythemia (ET) is diagnosed at a rate of about 2–3 per 100,000 individuals annually

(Tefferi 2012). The disease usually affects middle-aged to elderly individuals, with an average age at diagnosis of 50–60 years, although it can affect children and young adults as well (Hoffman et al. 2005). The pathologic basis for essential thrombocythemia is unknown, but essential thrombocythemia resembles PV in that cells of the megakaryocytic series are more sensitive to their normal growth factors. A mutation in the JAK2 kinase (V617F) has been found to be associated with essential thrombocythemia in around 40–50 % of cases (Levine et al. 2005). Aside from somatic mutations in *JAK2* and *MPL*, germline mutations in *TPO* may occur in cases of hereditary thrombocytosis. Hereditary thrombocytosis is rare. About 3–4 % of such cases go on to develop acute leukemia. Platelets derived from the abnormal megakaryocytes do not function properly, which contributes to the clinical features of bleeding and thrombosis.

Diagnosis

It is estimated that approximately half of the patients are asymptomatic at presentation. It is important to remember that ET is a diagnosis of exclusion; therefore, work-up to exclude other causes of thrombocytosis, both clonal and reactive, needs to be performed before a diagnosis of ET can be made. The most commonly used diagnostic criteria is the 2008 WHO classification (Table 9.3). Newly described somatic mutations of the *Calreticulin* (*CALR*) gene (insertions or deletions in exon 9) have been described in 67 % to 82 % of essential thrombocythemia and 88 % to 100 % of myelofibrosis patients who do not carry the JAK2 or MPL mutations. Patients with this mutation were found to have higher platelet counts and lower levels of hemoglobin compared to patients who have mutated JAK2. There is also a lower risk for thrombosis and longer overall survival for patients with *CALR* mutations. Idenitification of *CALR* mutations may have a role in the future for diagnosing and possibly prognosticating non JAK2 positive myeloproliferative disorders, especially essential thrombocythemia and myelofibrosis (Klampfl et al. 2013, Nangalia et al. 2013).

Treatment/Prognosis

The main cause of morbidity and mortality in ET patients is thrombotic events similar to PV patients. Therefore the main goal of therapy is to reduce thrombotic risks. This is accomplished by giving low-dose aspirin with or without a cytoreductive therapy. The same cytoreductive therapies used in PV are used for ET patients. The risk stratification scheme on whether to start cytoreductive therapy used in PV is also used for ET patients (Ruggeri et al. 1998). The main goal of cytoreductive therapy is to prevent thrombosis. Sometimes, cytoreductive therapy may be given in ET patients with low-risk disease but having vasomotor symptoms not responsive to aspirin or supportive measures. While on cytoreductive therapy, keeping platelet counts between 200,000 and 400,000/μl is appropriate. Aside from thrombotic risks, the elevated platelet counts particularly in cases of extreme thrombocytosis can also put patients with ET at risk for acquired vWF disease and bleeding (Reikvam and Tiu 2012). Measurement of ristocetin cofactor activity can help in the diagnosis, and cytoreductive therapy can help restore platelet counts to normal levels. *JAK* inhibitors are currently undergoing trials in ET patients. Factors associated with inferior survival in ET patients include advanced age (≥60 years old), elevated WBC (≥15,000/μl), smoking, prior venous thrombotic events, diabetes mellitus, and anemia (<12 g/dl in females and <13.5 g/dl in males) (Gangat et al. 2007).

Primary Myelofibrosis

General Features

The annual incidence of MF is estimated at 0.2 cases per 100,000 persons per year. The median age at diagnosis is 65 years old, and about 20 % of affected patients are younger than 55 years old. Primary myelofibrosis (PMF) is more common in males, Caucasians, and adults. However, in younger children, girls are affected twice as frequently as boys.

Myelofibrosis results in excessive bone marrow fibrosis and loss of hematopoietic cells, with subsequent marked increase in extramedullary hematopoiesis (primarily in the liver and spleen, which enlarge significantly). Myelofibrosis may be primary or secondary to a number of hematologic, malignant, and nonmalignant conditions.

In primary myelofibrosis, large numbers of nucleated RBCs (normoblasts) and granulocytes are released into the circulation (leukoerythroblastosis). These primary myelofibrosis progeny cells stimulate bone marrow fibroblasts (which are not part of the neoplastic transformation) to secrete excessive collagen. Bone marrow failure eventually occurs, with consequent anemia and thrombocytopenia. Malignant or acute myelofibrosis, an unusual variant, has a more rapidly progressive downhill course; this variant may actually be a true megakaryocytic leukemia. The molecular mechanisms that lead to myelofibrosis are not currently understood, but it has been postulated that myelofibrosis arises from somatic mutations of hematopoietic progenitor cells (Smith et al. 1990). Alternatively, mutations could result in aberrant stem cell niches forming in inappropriate locations and/or unfavorable conditions for hematopoietic expansion within the bone marrow. Thrombocytosis can be seen in MF patients although less commonly than in CML, PV, and ET.

Diagnosis

Most MF patients at presentation complain of either splenomegaly-related (early satiety, abdominal discomfort/pain) or cytokine-mediated (fatigue, night sweats, weight loss) symptoms. Splenomegaly is found in 80–85 % of patients. Most patients have anemia and normal platelet counts at presentation. Some patients have high WBC counts. Thrombocytosis is less frequent but when present can sometimes complicate the initial distinction between PMF and ET. However, the presence of leukoerythroblastic changes in the peripheral blood and the presence of characteristic BM changes, particularly extensive bone marrow reticulin fibrosis or collagenous fibrosis, will subsequently point towards a diagnosis of MF. Diagnosis is made in accordance with the 2008 WHO criteria (Table 9.3).

Treatment/Prognosis

The only known potential curative option for patients with MF is an allogeneic HCT. Other non-transplant therapies are primarily directed towards improvement of cytopenias, constitutional symptoms, and clinical manifestations related to extramedullary hematopoiesis. In the presence of extreme thrombocytosis, cytoreductive therapies used for PV and ET, and JAK inhibitors, if appropriate, can be used. Anemia can be managed with androgenic steroids, glucocorticoids, and immunomodulatory drugs (IMiDs) like lenalidomide and thalidomide with or without steroids. Some MF patients with anemia can benefit from erythropoiesis-stimulating agents, but these must be used with caution or avoided for patients with concomitant splenomegaly because of concern for worsening splenomegaly and even splenic rupture (Tefferi 2011; Tabarroki and Tiu 2012). For patients who have intermediate- and high-risk MF with cytokine-mediated and splenomegaly-related symptoms, JAK inhibitors like ruxolitinib can be used (Verstovsek et al. 2012; Harrison et al. 2012). Various risk stratification systems like the International Prognostic Scoring System (IPSS), Dynamic IPSS (DIPSS), and the DIPSS-plus are used to assess prognosis in patients with MF (Tefferi 2011).

Myelodysplastic Syndromes

Myelodysplastic Syndrome with Isolated Del(5q)

General Features

Although some estimates suggest 10,000–20,000 new cases each year in the United States alone, the incidence in patients over 70 may be as high as 15 cases per 100,000 per year (Aul et al. 2001). As with other forms of myelodysplastic syndrome (MDS), MDS with isolated del(5q) is predominantly a disease of the elderly; the median age of patients at the time of presentation is between 65 and 70 years. This type of MDS is found predominantly in females (Van den Berghe et al. 1974).

Cytogenetic studies demonstrate a deletion of the long arm of chromosome 5 (del(5q)) as the sole cytogenetic abnormality for this disease. Examination of bone marrow from patients harboring 5q deletions reveals numerous unilobular nucleated megakaryocytes, often marked by eccentric nuclei with copious granular cytoplasms exhibiting plentiful production of large platelets (Tinegate et al. 1983). Several genes localized to the long arm of chromosome 5q

influence hematopoiesis and could be involved in the pathogenesis of the syndrome, although a gene involving ribosome biogenesis, RPS14, has been implicated in the pathogenesis of this syndrome (Ebert et al. 2008). Down-regulation of RPS14 and additional ribosomal genes and genes involved in translation initiation, when coupled to the up-regulation of several proapoptotic genes, suggests that the 5q- syndrome represents a disorder of aberrant ribosome biogenesis. Although purely speculative, these abnormalities may lead to impairment of ribosome biogenesis and subsequent reduction of protein translation capacity, a defect that may be of particular importance for developing erythroid cells, whose survival and division require large amounts of protein synthesis.

Diagnosis

Most patients with this disease present with symptoms related to their anemia. In MDS with isolated del(5q), about 1/3 of patients will have thrombocytosis. A subset of patients with macrocytic anemia, normal pr elevated platelet counts, and BM erythroid hypoplasia are further classified as having "5q- syndrome." Both peripheral blood and bone marrow findings are taken into account when making this diagnosis (Table 9.3).

Treatment/Prognosis

MDS patients with isolated del(5q) are primarily treated with lenalidomide which results in excellent hematologic responses. Lenalidomide therapy usually leads to a decrease in the platelet counts of treated patients, although cytogenetic responses can also occur as a result of treatment with lenalidomide. Patients who fail to respond to lenalidomide can be managed using other MDS-related therapies like erythropoiesis-stimulating agents and hypomethylating therapies. The impact of thrombocytosis in the outcome of patients with MDS with isolated del(5q) is not clear, but the prognosis of patients with MDS with isolated del(5q) is generally good with a median survival estimated at 145 months and risk of AML transformation of <10 % (Swerdlow et al. 2008).

Myelodysplastic/Myeloproliferative Neoplasm

MDS/MPN-Unclassifiable (Refractory Anemia with Ring Sideroblast Associated with Marked Thrombocytosis)

General Features

Refractory anemia with ring sideroblast associated with marked thrombocytosis (RARS-T) is currently a provisional entity according to the 2008 WHO classification. It is included in the category of MDS/MPN-u. The exact disease incidence is not known. In one study, RARS-T accounted for 12 % of the total MDS and MDS/myeloproliferative neoplasm (MPN) cohort and 43 % of the MDS/MPN cohort (Tiu et al. 2011). The pathogenesis of RARS-T is not completely understood. Recurrent molecular mutations identified in RARS-T included *SF3B1* (72 %), *JAK2*^{V617F} (60 %), *MPL* (23 %), and *DNMT3A* (17 %) (Visconte et al. 2012a; Szpurka et al. 2006; Szpurka et al. 2010). Other mutations identified, although at lesser frequencies, involve *ASXL1*, *LNK,* and *TET2* genes (Visconte et al. 2012a). The thrombocytosis observed in some patients with RARS-T may be partly related to the presence of mutations in *JAK2* and *MPL*, while the pathogenesis of ring sideroblast formation in RARS and RARS-T can be explained by haploinsufficiency of the *SF3B1* gene (Szpurka et al. 2006; Visconte et al. 2012b). One study linked the presence of the SF3B1 mutation to an increased risk for blood clots (Visconte et al. 2012a).

Diagnosis

Thrombocytosis is an essential criterion in the diagnosis of RARS-T. Like other MDS and MDS/MPN, anemia and sometimes neutropenia can be present. Some patients may have hepatosplenomegaly. Another essential feature of the diagnosis is the presence of ≥15 % RS in the BM. There are no pathognomonic chromosomal findings in RARS-T (Table 9.3).

Treatment/Prognosis

Similar to other hematologic neoplasms, allogeneic HCT is a potentially curative option for RARS-T patients. Depending on the clinical manifestations of patients with RARS-T, treatments used for MDS like hypomethylating agents, IMiDs specifically lenalidomide, and erythropoiesis/granulocyte-stimulating agents can be used in patients with RARS-T. There are also no clear guidelines on how to manage thrombocytosis in these patients as the impact of thrombosis in these patients is not known. Low-dose aspirin therapy with or without cytoreductive therapy may be appropriate for patients at increased risk for arterial or venous thrombotic events although studies definitively supporting this recommendation are lacking.

Prognosis of patients with RARS-T are usually assessed using risk stratification systems like the IPSS, World Health Organization (WHO) Classification-Based Prognostic Scoring System (WPSS), or revised IPSS, although these prognostic scoring systems were not specifically designed for patients with RARS-T and probably only included a small subset of RARS-T patients (Greenberg et al. 1997; Malcovati et al. 2007; Greenberg et al. 2012).

Acute Myeloid Leukemia

Acute Megakaryoblastic Leukemia (M7 AML by FAB and AML Not Otherwise Specified by 2008 WHO Classification)

General Features

The incidence of acute leukemia is approximately 2.3 per 100,000 people per year. Acute megakaryoblastic leukemia (AML)-M7 is a rare subtype of leukemia and represents 1.2 % of cases of adult leukemia, compared to 3–10 % of childhood leukemia. AML-M7 is an uncommon disease, composing 0.5–1.2 % of newly diagnosed adult acute myeloid leukemias. Thrombocytosis occurs within this FAB subtype of AML (also known as acute megakaryoblastic leukemia or AMKL) but is uncommon (Dastugue et al. 2002). Although most AML-M7 patients present with cytopenia (including thrombocytopenia), elevated platelet counts could be observed in roughly 15 % of patients (Roumier et al. 1999). However, it should be noted that cases of extreme thrombocytosis up to 4,600,000/μl have been reported (Balatzenko et al. 2004).

AML-M7 frequently presents with cytopenias despite platelet counts often exceeding 100,000/μl in approximately 30 % of the patients (Peterson and Levine 1987). AML-M7 typically has a genetic component, most notably mutations in *GATA1* or *MKL1*, two transcriptional regulators of megakaryocytic differentiation (Orkin et al. 1998; Cheng et al. 2009). Because AML-M7 is characterized by higher incidence and complexity of cytogenetic abnormalities, chromosomal fusions and translocations have also been reported, mainly resulting in expansion of myeloid progenitor cells (Balatzenko et al. 2004; Min et al. 2011; Gu et al. 2007).

Diagnosis

The clinical features of AML-M7 patients are similar to other AML patients. Cytopenias are common (particularly anemia and thrombocytopenia), but thrombocytosis is sometimes noted. Hepatosplenomegaly is not a common feature. Like other AML, the diagnosis is primarily based on the identification of ≥20 % blasts in the bone marrow or the peripheral blood of which at least 50 % are of the megakaryocytic lineage. There are no unique chromosomal abnormalities associated with AML. However poor prognostic cytogenetic abnormalities such as inv(3) can be observed (Swerdlow et al. 2008).

Treatment/Prognosis

Treatment approaches are similar to other AML subtypes. Induction chemotherapy with cytarabine and an anthracycline-based therapy is the usual approach. Clinical trials are appropriate if available. Allogeneic HCT is potentially curative for these patients.

Clinical Vignette 5 (Continued)

The patient was found to have the BCR–ABL fusion gene by FISH, and the metaphase cytogenetics test performed from the bone marrow biopsy specimen shows t(9;22)(q34;q11).

The patient has a diagnosis of chronic myeloid leukemia, was BCR–ABL1 positive, and was started on nilotinib 300 mg PO BID. Dasatinib was not given because of the prior history of severe pleural effusions. A repeat CBC in 2 weeks showed a platelet count of 225,000/μL.

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Prolonged Bleeding After Surgery

10

Sam Schulman

Abbreviations

aPTT	Activated partial thromboplastin time
ASA	Acetylsalicylic acid
CBC	Complete blood count
CI	Confidence interval
DIC	Disseminated intravascular coagulation
NSAIDs	Nonsteroid anti-inflammatory agents
PBAC	Pictorial bleeding assessment chart
PCC	Prothrombin complex concentrate
PT	Prothrombin time
rFVIIa	Recombinant activated factor VIIa
TCT	Thrombin clotting time
VWD	von Willebrand disease

Clinical Vignette

A 14-year-old male has continued to bleed on and off for 10 days after tonsillectomy. He spits out clots and blood. He has taken aspirin 325 mg when needed for pain after the surgery. Hemoglobin has now dropped from 141 to 75 g/L and we are contemplating blood transfusion. Platelet count and prothrombin time (PT) are normal, activated partial thromboplastin time (aPTT) is borderline, 40 s. The patient does not have a history of bleeding or any family history thereof, except that his father required multiple transfusions after open-heart surgery.

Epidemiology

Bleeding after surgery or a procedure can be a normal occurrence, e.g., transient hematuria after transurethral prostate resection or collection of half a liter of blood in the drain after hip replacement. Increased bleeding during and after surgery can often be explained by *aggravating circumstances* (see examples below) and would then also be expected (Schulman et al. 2010).

- Extensive surgery
- Infection
- Tumor invasion
- Anomalous blood vessels
- Accidental operative vascular injury
- Revision surgery
- Concomitant antithrombotic medication
- Known congenital bleeding disorder
- Liver or kidney disease

The risk for unexpected bleeding after surgery varies widely depending on the type of procedure

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but also on the experience of the surgeon. In a systematic review of studies with patients who stopped their anticoagulant treatment for invasive procedures and either received or did not receive bridging anticoagulation with heparin, major bleeding occurred in the latter group in 18 of 2104 (0.9 %; 95 % confidence interval [CI], 0.2–1.6) and overall bleeding in 3.4 % (95 % CI, 1.1–5.8) (Siegal et al. 2012). The risk increases dramatically for emergency surgery as seen in an analysis of the population in the RE-LY study that compared dabigatran with warfarin for stroke prophylaxis in atrial fibrillation. Major bleeding was described here in 17.7–21.6 % of emergent procedures compared to 2.8–3.8 % after elective surgery (Healey et al. 2012). This could obviously reflect insufficient delay from the last dose of the anticoagulant drug. After adenoidectomy or tonsillectomy, major bleeding is observed in 0.5 % (Tomkinson et al. 2012) and any bleeding in approximately 5 % (Shargorodsky et al. 2012).

Definitions

The terms "major bleeding" or "prolonged bleeding" have until recently been very heterogeneous, even in the setting of clinical trials. An attempt to harmonize the definition of major bleeding was made through the International Society on Thrombosis and Haemostasis, both for medical patients (Schulman and Kearon 2005) and for surgical patients (Schulman et al. 2010). Any detailed definition of "prolonged" bleeding has been carefully avoided. It is thus up to the surgeon to determine whether the duration of bleeding was longer than expected under the circumstances.

Investigation: Medical History

For patients with unexpected postoperative bleeding, the investigation starts with a careful *review of the medical history*, which should include the following items:

1. Previous bleeding events
   (a) Spontaneous or after challenges
   - Tooth extractions
   - Major surgery, child delivery
   - Menstruation
   - If spontaneous—from mucosa, skin, muscle, and joint
     – Recent onset or lifelong history
     (b) Immediate or delayed onset after challenge
     (c) Requiring reoperation, transfusion, or other treatment
2. Renal or hepatic disease, hypothyroidism, and autoimmune disorder
3. Concomitant medications and dietary supplements
   (d) What is the patient using for pain?
4. Family history
   (e) First-degree relative or more distant
   (f) On father's or mother's side

Typical for a *disorder of the primary hemostasis* (platelet disorder or von Willebrand disease (VWD)) is the pattern of immediate onset and mainly mucosal bleeding and ecchymoses. Conversely, a *disorder of the secondary hemostasis* (coagulation factor defect) is characterized by a delayed onset and often internal bleeding, typically in muscles and joints, although skin bruising is also common. Spontaneous bleeding with recent onset can be a sign of development of autoantibodies against any component in the hemostatic system, whereas a lifelong history suggests a congenital deficiency. Delayed onset (or secondary) hemorrhage appears to be at least as common as early (primary) hemorrhage after tonsillectomy and is probably related to premature discharge of the eschar (Wei et al. 2000).

A first-degree relative with bleeding diathesis raises the suspicion of a hereditary bleeding disorder, whereas a second-degree relative on the maternal side and bleeding exclusively among males is almost pathognomonic for hemophilia A or B (deficiency of factor VIII or IX). A diagnosis of hemophilia is sometimes established in patients with a negative family history. The reason is either a de novo mutation or that the genetic defect has been passed on entirely by females for several generations (Peyvandi et al. 2006). De novo mutations are responsible for one third of the hemophilia cases.

It is important to note that a negative bleeding history by no means excludes a congenital bleeding disorder. Every now and then patients are diagnosed with the mild form of hemophilia at an advanced age, after their first surgical challenge or major trauma in life, as already reported in 1964 (Pappas et al. 1964). Postoperative bleeding in combination with a negative history for any bleeding despite surgical challenges could be either due to an acquired defect (medication, autoantibodies, consumption coagulopathy) or to local pathology.

Excessive bleeding is a highly subjective observation by patients, e.g., assessment of menorrhagia, where the patient often compares her experiences with her mother—who may have the same primary hemostatic disorder. It is often helpful to use a standardized questionnaire to try and quantify more objectively the bleeding diathesis (Rodeghiero et al. 2010) or specifically a pictorial bleeding assessment chart (PBAC) for suspected menorrhagia (Philipp et al. 2011). The sensitivity of the PBAC as a screening tool for hemostatic defects was 89 % in a recent study (Philipp et al. 2011).

Patients will, when questioned about their medications, at best provide an account for their prescriptional drugs but they will usually omit the over-the-counter medications. In order to capture sporadic use of acetylsalicylic acid (ASA) or nonsteroid anti-inflammatory agents (NSAIDs), the patients should be asked what they would use for headache and other pain. Low-dose ASA impairs the platelet function for about 3 days whereas 325 mg ASA inhibits the platelet aggregation for 7–10 days. For NSAIDs the duration of their platelet inhibition parallels their half-life, which is a few hours for ibuprofen and diclofenac and 1–2 days for naproxen. Clopidogrel and warfarin should be stopped for 5 days before surgery, though some surgeons prefer a longer duration off clopidogrel.

Physical Examination

This part of the investigation should include inspection of the skin. Large and palpable bruises are typical for coagulopathies. Petechiae are mainly a manifestation of vascular disorders on the capillary level but to some extent also a manifestation of platelet disorders.

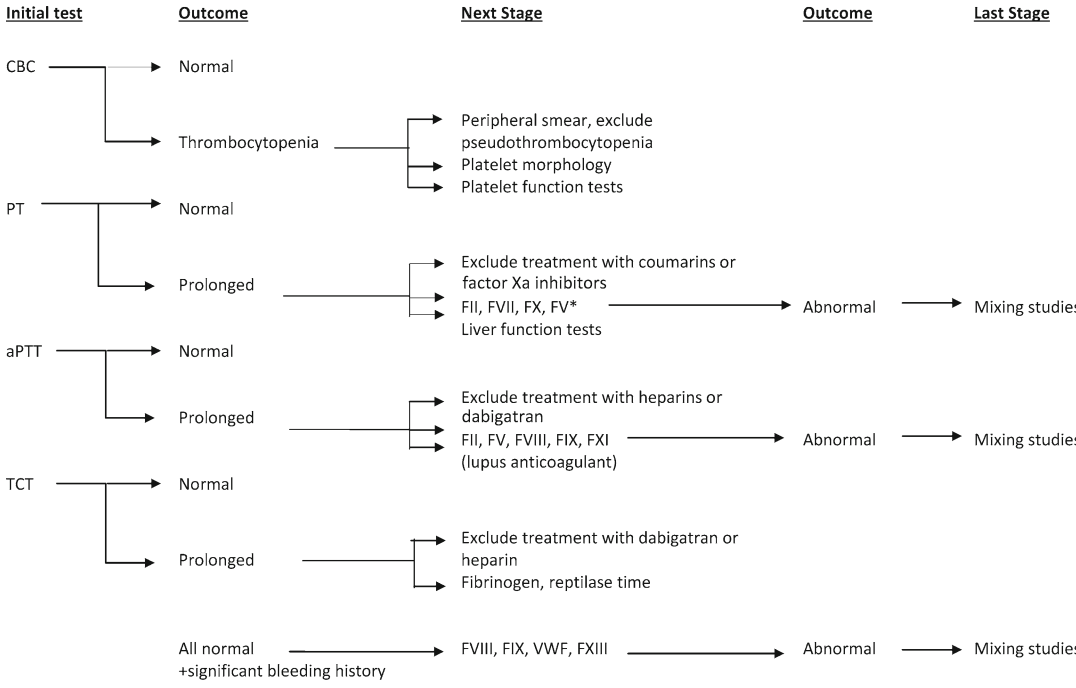
If the surgical area lends itself to inspection, it should be possible to assess whether there is rapid (pulsating) or slow diffuse bleeding and also whether the bleeding is confined to the surgical wound area or also has other localizations. The latter could indicate disseminated intravascular coagulation (DIC). Rapid bleeding could be due to arterial injury or from operation in an area with abnormal blood vessels, e.g., hemangioma or tumor.

The joints are also examined. Synovitis or a reduced range of motion are typical for hemophilia-associated arthropathy but can be seen with other coagulopathies. Conversely, hyperextension of the joints is a cardinal sign of Ehlers-Danlos collagen disorder.

Signs of liver or kidney disease should lead to further evaluation of the severity of the respective condition. Liver disease can be associated with the whole spectrum of bleeding manifestations since most of the coagulation factors are synthesized by the hepatocytes and are reduced in severe liver cirrhosis. The platelet count is also reduced by hypersplenism secondary to portal hypertension and this is often combined with a platelet function defect. In severe renal failure there is also a platelet function defect that has been ascribed to the blockage of surface receptors by uremic toxins such as guanidinosuccinic acid.

Laboratory Investigation

Preoperative screening for hemostatic disorders in asymptomatic patients is not helpful to predict bleeding events after surgery, as shown in a prospective French study with more than 3,000 patients (Houry et al. 1995) and in a systematic review (Eckman et al. 2003). In patients with a history of bleeding diathesis or in the acute situation with a patient with prolonged bleeding after surgery, the investigation should start with the simple screening tests: complete blood count (CBC), PT, aPTT, and thrombin clotting time (TCT). Thereafter, an algorithm can be used (Fig. 10.1).



*FV deficiency is only deleted with PT using the Quick method, which is the most disseminated, but not with the Owren method.

Fig. 10.1 Algorithm for laboratory investigation of postoperative bleeding

Platelet function tests should be performed at a specialized hemostasis laboratory with the blood samples obtained on site. There are instruments for global analysis of platelet function, such as PFA-100® (Siemens Healthcare Diagnostics, Deerfield, IL), but they are not very sensitive for the common, mild functional defects. Pseudothrombocytopenia is a laboratory artifact related to ethylenediaminetetraacetic acid (EDTA)-induced platelet clumping. This is usually revealed on a peripheral blood smear, and when the blood instead is drawn into a test tube with citrate as anticoagulant, the platelet count becomes normal. Several platelet function defects are combined with abnormalities in the platelet morphology, both regarding size and appearance.

In case of a low level of a specific coagulation factor, the laboratory should be asked to perform mixing studies. Patient plasma is then mixed with an equal volume of normal plasma and analyzed again with the screening test that was prolonged (PT or aPTT) without as well as after incubation at 37 °C for 30, 60, and/or 120 min. The interpretation of results is shown in Table 10.1.

Table 10.1 Interpretation of the screening test (PT or aPTT) in mixing tests

	Without incubation	After incubation
Isolated deficiency of a factor	Normalized screening test	Normalized screening test
Specific factor inhibitor	Normalized or prolonged screening test	Prolonged screening test
Lupus anticoagulant	Prolonged screening test	Prolonged screening test

A prolonged TCT in combination with a normal reptilase time identifies the presence of heparin in the sample. Dabigatran at therapeutic levels causes an excessive TCT.

If DIC is suspected, the platelet count will be reduced; the PT, aPTT, and TCT may be prolonged; and the fibrinogen level is low for the circumstances. Since fibrinogen is an acute phase reactant, levels within the reference range in a patient with sepsis

should be interpreted as abnormal. Fibrin D-dimer is high or very high in DIC.

Patients with the mild form of hemophilia A or B may have normal screening tests, and in case of clinical suspicion, the factor assays should be performed. The screening tests do not identify deficiencies of von Willebrand factor (vWF) in mild or moderate form or factor XIII or the components of the fibrinolytic system. Of these only VWD is common, with a prevalence of about 2 % in population studies.

Hyperacute Analyses

Abnormal bleeding during surgery requires immediate investigation in order to provide adequate therapy for hemostasis so that the procedure can be safely concluded. The screening tests that are performed by all hospital laboratories (platelet count as part of CBC, aPTT, PT, fibrinogen, and D-dimer) are helpful, but there is a delay that can result in suboptimal treatment while awaiting the results. Point-of-care analysis with thromboelastography, of which there are several variants, provides results within 10–15 min. The results inform the clinician on which component(s) of the hemostatic system is/are deranged and whether the bleeding is best treated with platelets, plasma (or factor concentrates), or antifibrinolytic agents. Several randomized studies have demonstrated that with the use of this type of instrument, the blood loss seems to be reduced and with significantly lower utilization of red cell, platelet, and plasma transfusions (Ak et al. 2009; Avidan et al. 2004; Cui et al. 2010; Shore-Lesserson et al. 1999; Westbrook et al. 2009).

Treatment Options

Local Hemostatic Measures

There are several options for local or regional hemostasis, as listed below:

- Compression
- Astringent agents (cold temperature, alum, adrenalin)
- Chemical barriers (gelatin, cellulose, collagen, inorganic films)
- Procoagulants (topical thrombin, fibrin sealant—a combination of fibrinogen and thrombin)
- Cauterization, argon plasma coagulation
- Interventional procedures such as coiling or arterial embolization

Potential side effects are tissue necrosis or formation of alloantibodies to components in the fibrin sealant, e.g., against factor V or thrombin. These antibodies may cross-react with the patient's own coagulation factor although this is a rare phenomenon (see Chap. 5).

General Supportive Measures

When the bleeding causes a rapid reduction of the circulating blood volume of at least 30 %, the patient becomes hypotensive. The American Association of Blood Banks suggests that the decision to transfuse *red cells* be based on both symptoms and the level of hemoglobin (Carson et al. 2012). They strongly endorse a restrictive transfusion policy for hospitalized and stable patients, using a threshold of 70–80 g/L. For patients with preexisting cardiovascular disease, a restrictive threshold of 80 g/L and occurrence of symptoms are suggested to trigger transfusion. The "symptoms" should be expanded to a "clinical picture," which includes patient age, cardiorespiratory signs and symptoms, mental status, and other signs of organ ischemia as well as the risk of further reduced oxygenation (Practice Guidelines 2006). Elderly patients with acute myocardial infarction seem to benefit from being transfused up to hemoglobin of at least 100 g/L (Hebert et al. 1999). It is also interesting to note that when the hemoglobin drops below this level, the platelet function becomes increasingly impaired. The reason is that platelets, instead of rolling along the endothelium and identifying damages, find room to move centrally in the blood flow.

Improved oxygenation can obviously also be provided by adequate ventilation and the use of oxygen. Other supportive measures include volume substitution and withholding or reversing any antithrombotic agent.

Systemic Hemostatic Treatments

Platelet transfusions are indicated in the following situations when there is bleeding (Practice Guidelines 2006):

1. Platelet count of less than 80–100/mL in case of life-threatening bleeding (e.g., central nervous system, other internal, and severe bleeding) or of less than 50/mL for major but less serious bleeding
2. Severe platelet dysfunction, even with normal platelet count
   - Congenital disorders such as Glanzmann's thrombasthenia or Bernard-Soulier's disease, where pharmacological agents may not work
   - Iatrogenic platelet dysfunction due to
     - Irreversible antiaggregants (clopidogrel, prasugrel, abciximab)
     - Hemodilution in massive transfusion

Patients with immune thrombocytopenia are often refractory to platelet transfusion. It has been discussed whether in DIC, platelet transfusions may actually aggravate the condition but this has not been proven. Thus, for patients with DIC, active bleeding and low platelet count (<50,000/μL) transfusion of platelets can be considered in conjunction with other corrective therapy and elimination of the cause of DIC.

The expected rise in platelet count of 30,000/μL after transfusion of six single donor units or one apheresis unit may not be seen in DIC, sepsis, immune thrombocytopenia, or splenomegaly due to consumption/sequestration.

Platelet transfusions do not seem to reverse bleeding caused by the P_2Y_{12}-receptor blocker ticagrelor, as long as the drug, which is active without any prior metabolic step, is present in the circulation.

Plasma transfusion is indicated when there is no information on a specific coagulation factor defect for which a purified concentrate is available or when there is a general deficiency of these factors due to poor synthesis (liver failure), dilution, or consumption (DIC). Abnormal global coagulations tests, such as aPTT and PT in a bleeding patient, support the need for plasma transfusion, whereas asymptomatic patients with such deranged tests rarely have an indication for replacement therapy. On the other hand, if a bleeding patient has normal aPTT and PT, plasma is unlikely to be effective.

For patients with congenital factor V deficiency, there is no specific concentrate and plasma should be given in case of hemorrhage. Conversely, for patients with bleeding in association with treatment with vitamin K antagonists, it is preferable to use a concentrate of the deficient factors—prothrombin complex concentrate (PCC), see below.

In massive transfusion (usually defined as ≥10 units red cells within 24 h) there is a high risk for dilution coagulopathy, but it is debated how early and in what internal proportion units of plasma and platelets versus red cells should be transfused to these patients. There is a recent tendency, based on positive observations in the military setting, to increase the proportion of these hemostatic blood components to *1 unit red cells/1 unit plasma/1 apheresis unit* (or six single donor units) platelets.

Plasma can be treated with viral inactivation, for example, using solvent-detergent, and this is commercially available in some countries.

Cryoprecipitate can be used for substitution of fibrinogen when specific fibrinogen concentrates are unavailable (see Fibrinogen below). A fibrinogen level of ≤100 mg/dL in a bleeding patient calls for treatment to raise the level (Practice Guidelines 2006). In addition, cryoprecipitate contains factor VIII, VWF, and factor XIII, but there are purified concentrates available for each of those factors. Cryoprecipitate is used at some hospitals for bleeding after cardiac surgery. The volume needed to replace fibrinogen is smaller than with plasma. Drawbacks of cryoprecipitate are the lack of viral inactivation and the relatively high risk of allergic reactions due to the low degree of purification from other plasma proteins.

Fibrinogen concentrate at a dose of 2 g has, in a small randomized controlled trial in patients with coronary artery bypass surgery with a preoperative level of <3.8 g/L, been shown to reduce postoperative blood loss (Karlsson et al. 2009). The concentrate is virally inactivated, and the risk of transfusion reactions is much smaller than with cryoprecipitate.

Table 10.2 Calculation of the dose of prothrombin complex concentrate based on the prothrombin time, expressed as international normalized ratio (INR)

Current INR	Target INR = 1.5	Target INR = 1.1–1.2
"Therapeutic" = 2–3.0	20 IU/kg	30 IU/kg
"High" = 3.0–5.0	30 IU/kg	40 IU/kg
"Extremely high" >5.0	40 IU/kg	50 IU/kg

Prothrombin complex concentrate (PCC) contains factors II, IX, and X (3-factor concentrate) and some of them also factor VII (4-factor concentrate), i.e., the vitamin K-dependent coagulation factors, which are reduced by coumarin anticoagulants in liver failure, DIC, and dilution coagulopathy. PCC is 20 times more concentrated than plasma and thus there is, as opposed to plasma transfusions, no risk for volume overload. This permits a more effective and much faster normalization of the PT. The required dose can be quite precisely estimated from the difference between the current and the desired PT and adjusted for the body weight (Schulman 2003). A simplified calculation strategy is shown in Table 10.2. The potential risk associated with PCC is thromboembolism, the risk for which in a meta-analysis was estimated at 1.4 % (95 % confidence interval (CI), 0.8–2.1 %) (Dentali et al. 2011). This event rate is, however, not significantly different from what is seen in patients who have their vitamin K antagonist treatment interrupted for surgery and receive bridging anticoagulation with heparin (0.9 %; 95 % CI, 0.0–3.4 %) (Siegal et al. 2012). It is thus possible that the unmasking of the underlying thrombogenic condition or the activation of coagulation by surgery or trauma is the major risk factor rather than PCC.

Recombinant activated factor VIIa (rFVIIa) is only indicated for hemophilia with inhibitors or certain severe platelet function disorders, such as Glanzmann's thrombasthenia (in Europe). But it has been evaluated and also used extensively for bleeding in patients without preexisting bleeding disorders. The results from clinical trials in patients with intracranial hemorrhage, trauma-related hemorrhage, and postpartum bleeding or in cardiac surgery have not been convincing regarding net benefit. Nevertheless, it is difficult for the hematologist to explain to the surgeon or anesthesiologist why this drug should not be used when there is massive hemorrhage and other measures have failed.

Problem areas include uncertainty regarding the optimal dose, since this is not an example of replacement to physiological levels. Typically, the standard dose for patients with hemophilia of 90 μg/kg is given and repeated after approximately 2 h. Second, there is no coagulation parameter that has demonstrated utility in monitoring the treatment or in predicting the effect. Third, a meta-analysis demonstrated an increased risk for arterial thromboembolic events versus placebo (odds ratio 1.68; 95 % CI, 1.20–2.36), which was pronounced in the elderly subpopulation and for the off-label indications (Levi et al. 2010).

Other *single factor concentrates* should be used for the respective factor deficiencies in case of bleeding or as prophylaxis before surgery. For factor II (prothrombin) deficiency and often also for factor X deficiency, PCC is used, although there is a factor IX-X concentrate available from one manufacturer (Factor IX Behring, Marburg, Germany). Some factor XI concentrates were associated with thromboembolic events (Bolton-Maggs et al. 1994), and the patients with congenital factor XI deficiency and bleeding may do best with a very low dose of rFVIIa (Schulman and Nemeth 2006). For patients with VWD and postoperative bleeding, a combined VWF-factor VIII concentrate is better than a pure VWF concentrate. The reason is that factor VIII becomes unprotected against and degraded by proteases in the absence of VWF. After infusion of pure VWF, there is a delayed increase in factor VIII, which also is needed to halt the bleeding.

Hyperfibrinolytic conditions are mostly treated effectively with *antifibrinolytic agents* such as tranexamic acid or epsilon aminocaproic acid. These conditions are seen with bleeding after pregnancy complications and prostate surgery. Importantly, prophylaxis with antifibrinolytic agents has been demonstrated to reduce bleeding after knee or hip replacement (Alshryda et al. 2011;

Gill and Rosenstein 2006), hip fracture surgery (Zufferey et al. 2010), spine surgery (Gill et al. 2008), and cardiac surgery (Henry et al. 2009). The dose of these agents has varied widely between trials. Although fibrinolytic activity mainly has been localized to mucosal membranes, the examples above highlight the efficacy of antifibrinolytics in a variety of organs.

Another synthetic agent that can improve hemostasis in a number of conditions is the vasopressin analogue d-arginine-deaminovasopressin (*desmopressin*), which acts by releasing VWF from the endothelium and also increases the levels of factor VIII as well as tissue-plasminogen activator to some extent. It has been demonstrated to reduce bleeding in:

- Many congenital platelet function disorders (not the most severe types)
- Acquired platelet function disorders due to
  - Aspirin, clopidogrel
  - Liver or kidney disease
- Mild form of VWD or hemophilia A
- Hemophilia A with inhibitor and a residual level of factor VIII

The dose is 0.3 μg/kg intravenously or subcutaneously and can be repeated after several hours. There is, however, a risk for tachyphylaxis, water retention, and hyponatremia after repeated dosing. Desmopressin is often combined with tranexamic acid to counteract further activation of fibrinolytic system.

Postoperative Bleeding Associated with Residual Anticoagulant Effect

Ideally, anticoagulants should have been held for a number of days before surgery, which is required to normalize hemostasis. In emergency surgery, this is not possible and knowledge as well as availability of effective reversal agents are of paramount importance (Schulman and Bijsterveld 2007). Patients with postoperative bleeding related to heparin should be treated with *protamine* sulfate, which neutralizes heparin by forming a stable salt complex. This works fully with unfractionated heparin whereas low-molecular-weight heparin is partly reversed. The dose is 1 mg per 100 units of circulating heparin and usually up to 50 mg is given at a time and may be repeated after 15 min, depending upon the PTT. When protamine is injected too fast, it can cause hypotension and bronchoconstriction due to release of histamine. It is better to give protamine sparingly since an excessive dose can result in platelet aggregation, consumption, and paradoxically increased bleeding.

In rare cases, when bleeding continues due to insufficient reversal of low-molecular-weight heparin (Lewis et al. 2001) or for fondaparinux-related bleeding (Lisman et al. 2003), *rFVIIa* seems to be effective.

Vitamin K antagonists should be reversed with *PCC* or, if unavailable, with rFVIIa or plasma. At the same time, a dose of *vitamin K* should be injected slowly intravenously to eliminate the risk for rebound when any of the above-mentioned agents wears off. That dose should be 1–2 mg only if the plan is to resume anticoagulation as soon as the bleeding has stopped or 5–10 mg when a longer lasting neutralization is desirable and anticoagulation will be resumed at a later stage.

The new oral anticoagulants have so far no antidotes. Ex vivo studies in human volunteers indicate that *PCC* may be effective to treat factor Xa inhibitor-associated bleeding (Eerenberg et al. 2011), although this remains to be verified in clinical situations. The oral thrombin inhibitor, dabigatran, is dialyzable (Stangier et al. 2010; Warkentin et al. 2012). It is also possible that activated PCC can reverse the effect of dabigatran (Dager and Roberts 2011; Marlu et al. 2012). Giving activated charcoal may help absorb dabigatran. Again more clinical data is desperately needed.

Practice Guidelines

Most of the published guidelines on management of postoperative bleeding are limited to one type of surgery or invasive procedure, to bleeding associated with a specific risk factor, or written in a non-English language. A task force of the American Society of Anesthesiologists published

a more general practice guideline in 2006 (Practice Guidelines 2006). The level of evidence is in many cases low, and it does not seem possible to provide exact criteria for transfusion of any of the blood components.

The case in the Clinical Vignette

The initially described young man had bled longer than what can be considered normal after tonsillectomy. He had started bleeding at home the first night after the surgery. Clearly, the pain medication with aspirin has contributed to the bleeding. The information that his father required transfusion after heart surgery is probably irrelevant, but there is a possibility that the patient has a mild form of an autosomal inherited coagulopathy. A normal PT and borderline aPTT exclude deficiency of factor VII. Further investigation with factor assays confirmed that the patient has hemophilia A in mild form with a factor VIII level of 15 % (0.15 IU/mL). Since this young patient did not demonstrate signs of poor oxygenation, blood transfusions were not given. The bleeding stopped after infusion of factor VIII concentrate to reach a level of 70 %. It was combined with tranexamic acid, 20 mg/kg orally every 8 h for 5 days. Two more doses of factor VIII were given. Desmopressin might not have been sufficiently effective in this case, requiring more than a threefold rise from the baseline factor VIII level. A patient with a new diagnosis of hemophilia should be referred to a hemophilia treatment center for registration, education, and follow-up.

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The Excessively Clotting Cancer Patient

11

Marcelo P. Villa-Forte Gomes

Abbreviations

ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
CNS	Central nervous system
DVT	Deep venous thrombosis
GI	Gastrointestinal
GU	Genitourinary
IU	International units
IVC	Inferior vena cava
LMWH	Low-molecular-weight heparin
NSCLC	Non-small cell lung cancer
PE	Pulmonary embolism
SC	Subcutaneously
SCLC	Small-cell lung cancer
TF	Tissue factor
UFH	Unfractionated heparin
VKA	Vitamin K antagonist
VTE	Venous thromboembolic disease

Clinical Vignette

A 62-year-old white male presented with rapidly progressive memory impairment and expressive aphasia, but no focal motor deficits or seizures. His past medical history was unremarkable. Brain MRI showed a 5 cm left temporal mass with involvement of the dura and leptomeninges. He underwent extensive surgical resection, and final pathology revealed gliosarcoma. He was started on radiation therapy and temozolomide. Repeat MRI several weeks later revealed further enhancement in the area of previous resection. Within 2 weeks of that new MRI finding, he developed sudden onset of left leg pain and edema. Venous duplex ultrasound of the lower extremities revealed acute deep vein thrombosis (DVT) involving the left femoral, popliteal, and calf (posterior tibial) veins. He was started on anticoagulation therapy in the form of low-molecular-weight heparin (LMWH) monotherapy: enoxaparin, 1 mg/kg subcutaneously (SC) every 12 h. Five months later, after four cycles of temozolomide, repeat brain MRI revealed disease progression with abnormal enhancement in the left temporoparietal region. His therapy was switched to *cis*-retinoic acid, and he enjoyed both clinical and radiological improvement. Because of residual disease and ongoing *cis*-retinoic acid therapy, and despite of his overall clinical/neurological improvement, he was kept on long-term anticoagulation therapy. However, instead of twice-daily enoxaparin, his therapy was switched to once-daily dalteparin at a dose of 150 international

(continued)

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units (IU) per kilogram (kg) SC once daily. He remained on such therapy for a total of 18 months, after which he grew tired of injections and requested a change to warfarin. After a total of two and a half years of anticoagulation therapy, *cis*-retinoic acid therapy was discontinued. Brain MRI showed no abnormal enhancement about the resection cavity and no recurrent disease. At that time, anticoagulation therapy was discontinued as well.

Introduction

Cancer is a well-defined hypercoagulable state that leads to an increased risk of venous thromboembolism regardless of location, histological type, or staging (Luzzatto and Schafer 1990; Kakkar et al. 1970). Cancer also increases the risk of arterial thromboembolism, but this association has not been as extensively studied as the risk of acute DVT and acute pulmonary embolism (PE). The interaction between malignant diseases and venous thromboembolic disease (VTE) has been reported since 1864, when Armand Trousseau first described the association of *thrombophlebitis migrans* and cancer in a patient with gastric carcinoma (Illtyd and Matheson 1935). VTE is a common complication in patients with cancer, and the incidence of VTE is higher in patients with advanced stage disease compared to those with more limited disease (Blom et al. 2005). The risk of VTE also varies depending upon the organ(s) involved, histological type, and chemotherapy or adjunctive therapy regimens employed (Kakkar et al. 1970; Blom et al. 2005; Levitan et al. 1999; Sarasin and Eckman 1993; Noble and Pasi 2010). Comorbidities such as heart failure and chronic obstructive pulmonary disease, which themselves are independent risk factors for VTE (Alikhan et al. 2003), may further increase the cancer-associated VTE risk. Compared to patients with nonmalignant diseases, patients with active underlying malignancy have higher rates of postoperative VTE, higher rates of recurrent VTE after anticoagulant therapy is discontinued, higher rates of recurrent VTE during similar regimens and intensity of anticoagulation, as well as higher rates of hemorrhagic complications during anticoagulant treatment. Development of VTE may represent the earliest clinical manifestation of an occult malignancy or the first clinical manifestation of recurrent cancer in patients who are deemed to have no evidence of disease. In addition, the occurrence of VTE in a patient with active cancer represents a poor prognostic factor in terms of disease staging and survival. Thus, not only are the rates of initial and recurrent VTE higher in patients with cancer than in those without (hence the term "hypercoagulability of malignancy"), but the development of acute VTE in the setting of active malignancy is also associated with more widespread disease and shorter survival.

Epidemiology of Cancer-Associated VTE

The incidence of symptomatic VTE in patients with cancer has been reported to be as low as 3.8 % and as high as 30.7 % (Luzzatto and Schafer 1990), corresponding to an estimated relative risk increase of 1.8- to 12-fold compared to the general population (Luzzatto and Schafer 1990; Blom et al. 2005; Heit et al. 2000). This wide variation in VTE rates can be explained by differences in study design, endpoints, population, and duration of follow-up (Khorana 2012). Rates of VTE are typically higher in studies that predefined VTE as a primary or secondary endpoint than in studies which collected data about VTE retrospectively and/or as part of drug complications and toxicity review analyses (Khorana 2012). Rates of VTE are higher in studies that included incidental (i.e., asymptomatic) VTE as opposed to symptomatic VTE only. Rates of VTE are also higher in studies that employed active rather than passive surveillance (Reynolds et al. 2008). Indeed, a meta-analysis of studies that incorporated an active VTE surveillance

protocol revealed that incidence rates of VTE were up to 50 times higher when active surveillance was employed, compared to passive surveillance alone (Reynolds et al. 2008).

The wide variability of VTE rates reported in the literature is also, in part, a consequence of lack of standardized definitions. Rates of VTE are higher in studies that have included intra-abdominal and visceral venous thrombotic events, as opposed to those that reported exclusively on the rates of acute lower- and/or upper-extremity DVT with or without acute PE. Moreover, contemporary studies have also utilized a broader definition of clinical thromboembolic events, which encompasses all VTE events—incidental (asymptomatic), symptomatic, and those involving any deep vein of the body—as well as arterial thromboembolism (De Stefano et al. 2005; Maraveyas et al. 2012). Such definition, while not uniformly adopted, is more comprehensive and results in higher rates of cancer-related thromboembolism when utilized as a formal study endpoint (Khorana 2012). In addition, evolving data on the clinical impact of incidental and/or asymptomatic VTE suggests that they may have a significant—if not similar—impact on mortality and VTE recurrence, when compared to symptomatic VTE events (Menapace et al. 2011; O'Connell et al. 2006, 2011).

Variables inherent to the different study populations and durations of follow-up also contribute to the somewhat discrepant VTE rates reported in the literature. Higher VTE rates have been reported out of North America than Asia, as well as from retrospective studies (Khorana 2012). Shorter duration of follow-up results in lower reported rates of VTE, and this was well illustrated by two studies that attempted to validate a formal clinical risk score to assess VTE risk in cancer patients (Khorana et al. 2008; Murray 2005). The study with shorter follow-up period (approximately 2.5 months) reported a 7 % rate of VTE in cancer patients who were deemed "high risk" for VTE (Khorana et al. 2008), whereas the study with longer follow-up period (6 months) reported a rate of VTE of 18 % (Murray 2005).

Despite the much higher VTE rates in cancer patients (compared to those without malignancy), the true incidence of VTE in patients with cancer is likely underestimated. Indeed, *postmortem* studies have reported VTE prevalence rates as high as 50 % (Luzzatto and Schafer 1990). It is also noteworthy that VTE is the second most common cause of death in patients with cancer, second only to infections and sepsis (Ambrus et al. 1975).

Among cancer patients, those with a previous history of VTE are 6–7 times more likely to develop new VTE than those without such a history (Samama 2000). Older age, male gender, and presence of central venous access devices also increase the risk of VTE in cancer patients (Khorana 2012; Deitcher et al. 2004; Khorana et al. 2007).

The hypercoagulable state of cancer is also expressed clinically by an increased risk of postoperative VTE. The incidence of postoperative symptomatic VTE in patients undergoing major surgery for malignancy has been reported as ranging from 22 to 52 %, which represents an estimated 1.5- to 3.6-fold increased risk of postoperative VTE compared to noncancer patients undergoing similar major surgical interventions (Piccioli et al. 1996). Rates of postoperative DVT have been reported as ranging between 3 and 25 % in patients undergoing surgery for malignant glioma (Marras et al. 2000). Thirty-day VTE rates (after hospital discharge) have been reported as 3.9, 7.5, and 19 % for individuals undergoing craniotomy for nonmalignant diseases, primary central nervous system (CNS) tumors, and metastatic disease to the brain, respectively (Chan et al. 1999). The incidence of fatal postoperative acute PE in patients undergoing major surgery for cancer is four times higher than in patients undergoing surgery for nonmalignant illnesses (Verso and Agnelli 2012; Agnelli 1997). The postoperative prothrombotic risk in patients with cancer persists for at least 4 weeks (Verso and Agnelli 2012) and perhaps as long as 3 months after surgery.

Not only is the risk of VTE higher in cancer patients, but the severity of cancer-related hypercoagulability appears to be greatest early in the course of the disease. A large population-based, case-control study demonstrated that the estimated

relative risk of VTE was highest within the first 3 months following the diagnosis of a malignancy, with an adjusted odds ratio (OR) of 53.5 (95 % CI, 8.6–334.3) compared to controls without malignancy (Blom et al. 2005). In that study, the estimated risk of VTE remained very high between 3 months and 1 year after the diagnosis of malignancy (adjusted OR = 14.3; 95 % CI, 5.8–35.2), decreasing progressively over time and becoming nonstatistically significant beyond 10 years after the diagnosis of cancer (Blom et al. 2005). Similar findings have been reported in patients with hematologic malignancies. In a prospective study of nearly 400 patients with acute leukemias, the incidence of thromboembolic events (both VTE and arterial thromboembolism) was 6.3 %, and all events occurred within 6 months of the diagnosis of leukemia (De Stefano et al. 2005). In a large retrospective, population-based study of more than 5,000 patients with acute leukemias, the 2-year cumulative incidence of VTE was 5.2 % for acute myeloid leukemia (AML) and 4.5 % for acute lymphoblastic leukemia (ALL), with the vast majority of events occurring within the first months following the diagnosis (Ku et al. 2009). Studies reporting on the association of VTE and lymphomas also showed that most VTE events tend to occur within 3–6 months of treatment (Elice and Rodeghiero 2012).

Although adenocarcinoma of the gastrointestinal (GI) tract has been traditionally considered the most "hypercoagulable" of all malignancies (a perception that dates back to Trousseau's original reports in the mid- to late 1800s), a Medicare claims data analysis revealed that the three neoplastic disease types with the highest VTE rates (above 100 cases of DVT/PE per 10,000 persons) included ovarian (120/10,000), brain (117/10,000), and pancreatic (110/10,000) cancers (Levitan et al. 1999). Other studies have reported similar findings, with cancers of the ovaries, brain, pancreas, stomach, and liver consistently displaying the highest association with VTE events, followed by cancer involving the lungs and kidneys (Sørensen et al. 1998, 2000; Baron et al. 1998; Murchison et al. 2004; Khorana and Connolly 2009). Rates of VTE may differ even between different histological types of disease involving the same organ, as illustrated by the fact that the incidence of VTE in patients with non-small cell lung cancer (NSCLC) is twice as high as that in patients with small-cell lung cancer (SCLC), albeit with identical poor prognosis (Chew et al. 2008).

Even though "solid" cancers have long been viewed as more "prothrombotic" than hematologic malignancies (which were once thought to be relatively "inert" from a thrombotic standpoint), this long-held concept does not appear to be true. In fact, Medicare data showed that VTE rates associated with lymphomas (98/10,000) and leukemias (81/10,000) were similar to those seen with gastric (85/10,000) and renal cell carcinoma (84/10,000) and even higher than the VTE rates seen with colorectal, genitourinary (GU), pulmonary, breast, hepatic, and head/neck malignancies (Sarasin and Eckman 1993). In addition, a large Dutch case-control study demonstrated that patients with hematologic malignancies (all lymphomas and leukemias considered together as a group) had the highest risk of VTE (adjusted OR, 28.0; 95 % CI, 4.0–199.7), followed by lung and GI malignancies (Blom et al. 2005), whereas a Scottish population-based cohort study found that lymphomas and ovarian cancers had the highest standardized incidence ratios following a new diagnosis of VTE (Murchison et al. 2004). Reported rates of thromboembolic events (VTE and arterial thrombosis) in patients with acute leukemias, lymphomas, and multiple myeloma range from 1.5 to 12.1 % (Elice and Rodeghiero 2012).

Extension of disease has also been shown to correlate with VTE risk. The prevalence of cancer-associated thrombosis has been reported to be as high as 50 % in palliative care inpatients (Johnson et al. 1999). Data from the Multiple Environmental and Genetic Assessment (MEGA) case-control study revealed that cancer patients with distant metastases had a 20-fold and a 58-fold increased risk of VTE compared to those without distant metastases and without malignancy, respectively (Blom et al. 2005). In another study, using population-based data from Danish Registries, 44 % of patients who had cancer diagnosed at the very same time of acute VTE already

had evidence of distant metastatic disease, compared with 35 % of control patients with cancer who did not have VTE, matched for type of cancer, age, gender, and year of diagnosis (Sørensen et al. 2000). In addition, patients with monoclonal gammopathy of undetermined significance (MGUS) have two- to threefold higher rates of VTE compared to the general population, yet the rates of VTE in patients with MGUS are only about one-third of those seen in patients with multiple myeloma (Kristinsson et al. 2008).

Remarkably, the hypercoagulable state of cancer may be heightened exponentially when chemotherapy is employed (Kakkar et al. 1970; Blom et al. 2005; Sarasin and Eckman 1993; Noble and Pasi 2010; Heit et al. 2000; Elice and Rodeghiero 2012; Khorana et al. 2005). Chemotherapy, in general, seems to increase the risk of VTE beyond what is expected from the disease itself. Specific chemotherapy agents, and even adjuvant hormonal therapies, can also lead to incremental hypercoagulability. This observation was originally described in patients with breast cancer: in those patients, the risk of VTE is exponentially increased when chemotherapy is employed (Sarasin and Eckman 1993; Heit et al. 2000; Levine et al. 1988) and is even higher when patients undergo combined treatment with chemotherapy and adjuvant hormonal agents such as tamoxifen, raloxifene, and aromatase inhibitors (Deitcher and Gomes 2004). Patients with advanced gastroesophageal cancer treated with cisplatin-based regimens have been shown to have twice as high VTE rates as those treated with oxaliplatin-based regimens (Starling et al. 2009). Bevacizumab-based regimens, as well as chemotherapy containing sunitinib or sorafenib, appear to increase the risk of arterial thromboembolic events but not that of VTE (Scappaticci et al. 2007; Nalluri et al. 2008; Choueiri et al. 2010). In a meta-analysis of thrombotic complications in children with ALL, those treated with a combination of asparaginase and prednisone had the highest risk of VTE, with regimens using prednisone carrying a higher risk of VTE than regimens that used dexamethasone (Elice and Rodeghiero 2012; Caruso et al. 2006). In a retrospective study including more than 1,000 patients with lymphomas, 72 % of all VTE events occurred while on chemotherapy, whereas 17 and 11 % of VTE events occurred before initiation and after completion of chemotherapy, respectively (Mohren et al. 2005). But arguably, the greatest impact of chemotherapy on the rates of VTE has been observed after the introduction of combined regimens of thalidomide or lenalidomide with dexamethasone or doxorubicin for treatment of multiple myeloma (Elice and Rodeghiero 2012). Available data indicates that the lowest rates of VTE in association with multiple myeloma have been described in patients treated with thalidomide or lenalidomide as single agents, while the combination of either of those drugs with dexamethasone increases VTE rates exponentially (Elice and Rodeghiero 2012; Cavo et al. 2004; Zangari et al. 2001). The highest rates of VTE observed during treatment for multiple myeloma were reported in association with regimens combining thalidomide and doxorubicin (Elice and Rodeghiero 2012; Zangari et al. 2002).

Because chemotherapy-associated hypercoagulability clearly compounds with the hypercoagulability of malignancy itself, this may very well explain why VTE rates tend to be highest in the first few months after the diagnosis of malignancy, when patients are most likely to have central venous access placement and receive chemotherapy.

While the hypercoagulability of malignancy can be overwhelmingly demonstrated by the increased rates of VTE in patients with established cancer, multiple observational studies have also demonstrated that VTE may be the presenting feature of an occult malignancy (Aderka et al. 1986; Cornuz et al. 1996; Monreal et al. 1991; Prandoni et al. 1992; Nordström et al. 1994; Bastounis et al. 1996; Monreal et al. 1997; Rajan et al. 1998; Hettiarachchi et al. 1998). This is illustrated by the fact that the risk of a new diagnosis of cancer is higher in individuals who present with unprovoked VTE but do not have any known diagnosis of cancer at the time of VTE diagnosis. Indeed, those studies revealed that 4–34 % of patients who present with "idiopathic" or unprovoked VTE will be diagnosed with can-

Table 11.1 Incidence of new diagnosis of cancer following an episode of venous thromboembolism

Study	Idiopathic	Situational	Controls
Aderka et al. (1986)	34.0	4.0	–
Monreal et al. (1991)	22.6	6.1	–
Prandoni et al. (1992)	10.5	1.9	–
Nordström et al. (1994)	11.0	–	7.0
Bastounis et al. (1996)	25.0	4.0	–
Monreal et al. (1997)	12.4	1.8	–
Rajan et al. (1998)	8.6	7.1	–
Hettiarachchi et al. (1998)	7.3	1.6	–

Data expressed as percentage (%)

cer within 12–24 months after their diagnosis of VTE, compared to only 1.6–7.1 % of those who present with a "situational" or provoked VTE event (Table 11.1) (Sørensen et al. 1998; Baron et al. 1998; Murchison et al. 2004; Aderka et al. 1986; Monreal et al. 1991; Prandoni et al. 1992; Nordström et al. 1994; Bastounis et al. 1996; Monreal et al. 1997; Rajan et al. 1998, Hettiarachchi et al. 1998). The risk of being diagnosed with cancer is highest within 12 months of VTE diagnosis (Sørensen et al. 1998; Baron et al. 1998; Murchison et al. 2004).

Mechanisms of Cancer-Related Hypercoagulability

While the so-called Virchow's triad described stasis of blood flow, vascular injury, and "changes in the blood itself" as seminal components for the development of VTE (Dickson 2004), in no clinical situation other than cancer are those three variables so intrinsically present. For example, renal cell carcinoma increases the risk of thrombosis because of direct renal vein invasion (vascular injury), renal vein and inferior vena cava (IVC) obstruction (venous stasis), and enhanced expression of tissue factor (TF) (hypercoagulability).

Multiple pathophysiological mechanisms by which cancer cells lead to clinical hypercoagulability have been proposed and/or demonstrated, including tumor-, leukemic blast-, and monocyte-derived TF; release of TF-bearing microparticles; tumor-associated hyperviscosity; direct vascular endothelial invasion; extrinsic venous compression; tumor cell expression of phospholipids which can support activation of prothrombin; tumor-mediated platelet activation; tumor- and circulating leukemic blast-derived "cancer procoagulant" (a cysteine proteinase that directly activates factor X); mucinous adenocarcinoma-derived sialic acid which supports the nonenzymatic activation of factor X; tumor-induced increase in levels of plasma fibrinogen, factor VIII, von Willebrand factor, plasminogen-activator inhibitor-1 (PAI-1), thrombin activatable fibrinolysis inhibitor (TAFI), and C-reactive protein; presence of immunoglobulins with lupus anticoagulant activity; tumor-associated elevated titers of immunoglobulin IgG anticardiolipin antibodies; tumor- and chemotherapy-related (acquired) deficiencies of natural anticoagulants; acquired activated protein C resistance (APC-R); tumor-induced disseminated intravascular coagulation (DIC); impaired fibrin polymerization due to high levels of immunoglobulins which impair fibrinolysis by interfering with the binding sites of plasmin and factor XIII; and abnormal fibrin which is resistant to fibrinolytic activity of plasmin (Elice and Rodeghiero 2012; Deitcher 2003; Meehan et al. 1995; Rong et al. 2005; Yu et al. 2005; Deitcher et al. 1994, 1996, 2003, 2006; Falanga et al. 1988; Athale and Chan 2003; Elice et al. 2006; Boccaccio et al. 2005; Deitcher and Crowe 2004; Zwicker et al. 2009; Smith and La Celle 1982; Palumbo et al. 2008, Wang et al. 2012; Prandoni et al. 1999; Ottinger et al. 1995; Edwards et al. 1993; Falanga and Donati 2001; Gordon 1992; Rickles et al. 1995; Bevilacqua et al. 1986; Beer et al. 2002; Petralia et al. 2005; Muir et al. 2003; Shaw et al. 2001; Keung et al. 1996; Stasi et al. 1993; Sampson and Kakkar 2002; Rickles et al. 2003; Palumbo et al. 2000, 2003; 2005). While these mechanisms are not universally present in every histological type of disease nor in every patient with malignant diseases, they do represent potential areas for ongoing research and targeted therapies (Table 11.2).

Table 11.2 Mechanisms of cancer-related hypercoagulability

Tumor-derived tissue factor (TF)
TF-bearing microparticles
Tumor-associated hyperviscosity
Direct vascular injury
Extrinsic venous compression
Tumor cell expression of lupus anticoagulant-type activity
Tumor-mediated platelet activation
"Cancer procoagulant"
Direct activation of factor X by mucinous adenocarcinoma-derived sialic acid
Impaired fibrinolysis
Tumor-induced disseminated intravascular coagulation
Acquired deficiencies of natural anticoagulants
Acquired activated protein C resistance (APC-R)
Increased plasma levels of fibrinogen, factor VIII, von Willebrand factor, and PAI-1

Natural History of Cancer-Associated VTE

Cancer patients with VTE are more likely to develop acute proximal (i.e., above-the-knee) DVT as well as to have greater thrombus burden, lower venous recanalization rates (based on contrast venography), greater rates of DVT propagation, and greater rates of VTE recurrence compared to patients with VTE who do not have an underlying cancer (Heit et al. 2000; Prandoni et al. 1996; The Columbus Investigators 1997; Bona et al. 1997; Douketis et al. 2000; Hansson et al. 2000; Prandoni et al. 2002; Hutten et al. 2000). In studies of postoperative pharmacological prophylaxis against VTE in cancer patients, reported rates of DVT objectively diagnosed by contrast venography range from 40 to 80 %, and the rates of symptomatic proximal DVT range from 10 to 20 % (Verso and Agnelli 2012). These rates of femoropopliteal DVT are higher than those reported after orthopedic, bariatric, or abdominal surgeries for nonmalignant illnesses (Gould et al. 2012; Falck-Ytter et al. 2012).

Cancer patients have a two- to fivefold increased relative risk of recurrent VTE compared to patients without cancer (Table 11.3) (Heit et al. 2000; Prandoni et al. 1996; The Columbus Investigators 1997; Bona et al. 1997; Douketis et al. 2000; Hansson et al. 2000; Prandoni et al. 2002; Hutten et al. 2000). In addition, a rate of recurrent VTE of 32 % has been reported in cancer patients who undergo IVC filter placement (Elting et al. 2004), which is much higher than the rates of IVC filter-related recurrent VTE published in a prospective study of IVC filter placement (Decousus et al. 1998).

Table 11.3 Recurrent VTE in patients with and without active malignancy

	Recurrent VTE		
Study	Noncancer[a]	Cancer[a]	Relative risk
Prandoni et al. (1996)	4.7	10.3	2
Hansson et al. (2000)	–	–	2
Douketis et al. 2000	–	–	3
Hutten et al. (2000)	9.0	27.1	3
Prandoni et al. (2002)	6.8	20.7	3

[a]Data expressed as percentage (%)

The risk of recurrent VTE is also influenced by the extent of disease, with VTE rates of 14.5, 44.1, and 54.1 % reported in cancer patients with less extensive, moderately extensive, and extensive disease, respectively (Hutten et al. 2000). Those rates correspond to a relative risk increase of VTE recurrence from approximately twofold in patients with limited disease to fivefold in those with moderate or extensive disease (Hutten et al. 2000).

But perhaps the greatest conundrum in the management of cancer patients with VTE is that not only do these patients have a greater risk of recurrent VTE, but they also have a greater risk of major hemorrhage while on oral anticoagulation therapy with a vitamin K antagonist (VKA) than patients without active cancer (Prandoni et al. 2002; Hutten et al. 2000).

In an Italian prospective cohort study ($n = 842$), a total of 181 patients had known cancer at entry in the study (Prandoni et al. 2002). The cumulative incidence of recurrent VTE was 20.7 and 6.8 % in patients with and without cancer, respectively, for a hazard ratio of 3.2 (95 % CI, 1.9–5.4). The cumulative incidence of major bleeding was 12.4 and 4.9 % in patients with and without cancer,

respectively, for a hazard ratio of 2.2 (95 % CI, 1.2–4.1) (Prandoni et al. 2002). The highest risk of VTE recurrence was observed in patients with cancer involving the lungs, followed by GI tract, brain, and GU tract, whereas the highest risk of major hemorrhage while on oral anticoagulation therapy was observed in patients with cancer of the GU tract. These findings could not be explained by any significant differences between cancer and noncancer patients in terms of (a) extent of the original VTE event; (b) proportion of patients presenting with acute PE as the index VTE event; (c) proportion of patients treated initially with unfractionated heparin (UFH) or LMWH, prior to conversion to an oral VKA; and (d) quality of oral anticoagulation (i.e., time in therapeutic INR range). Indeed, 83 % of all recurrent VTE events in cancer patients occurred when their INRs were within or above the therapeutic target range of 2.0–3.0. Conversely, a smaller proportion of patients with cancer had supra-therapeutic INR values (>3.0) at the time of bleeding (Prandoni et al. 2002). Both VTE recurrence and major bleeding occurred predominantly during the first month of oral anticoagulant therapy. In addition, the observed risks of VTE recurrence and bleeding while on anticoagulation were greatest in those with more extensive malignant disease, thus demonstrating that patients who are exquisitely hypercoagulable with advanced malignancy are also those in whom the risk of bleeding associated with oral anticoagulation therapy is highest.

Another study performed a retrospective analysis of the INR-specific incidence of recurrent VTE and major hemorrhage while on oral anticoagulation for patients with and without cancer who had been prospectively included in two randomized trials comparing initial VTE treatment with UFH versus LMWH (The Columbus Investigators 1997; Hutten et al. 2000; Koopman et al. 1996). There were 264 patients with cancer among 1,303 eligible patients (Hutten et al. 2000). The overall incidence of recurrent VTE was 27.1 events per 100 patient-years versus 9.0 events per 100 patient-years in patients with and without cancer, respectively, whereas the overall incidence of major hemorrhage was 13.3 events per 100 patient-years in cancer patients versus 2.1 events per 100 patient-years in patients without malignancy (Hutten et al. 2000). The statistically significant differences between patients with and without cancer could not be explained by differences in the percentage of time spent within the INR target range. Patients with active malignancy were three times more likely to develop recurrent VTE with INR values within therapeutic target range of 2.0–3.0 and also three times more likely to develop recurrent VTE with supra-therapeutic INR values, when compared to patients without malignancy (Hutten et al. 2000). Those findings meant that true "warfarin failure" occurred in almost 38 % of patients with active cancer. And the hypercoagulability of malignancy became even more apparent when the INR was subtherapeutic: cancer patients had 54.0 recurrent VTE events per 100 patient-years when their INR declined to <2.0, compared to only 15.9 events per 100 patient-years among patients without malignancy (Hutten et al. 2000).

Impact of VTE on Cancer-Related Outcomes

In patients with cancer, the occurrence of VTE is associated with poorer survival. In a Medicare claims data analysis, the probability of death within 6 months of initial hospitalization for acute VTE was twice as high in patients with malignant disease who were diagnosed with VTE compared to those with established malignancy but without a diagnosis of VTE (Levitan et al. 1999). In a population-based study from Danish Registries, the 1-year survival rate for patients who had cancer diagnosed at the time of the initial diagnosis of VTE was only 12 %, compared to 36 % in matched control patients with cancer but without VTE (Sørensen et al. 2000). Another study, which included patients in hospice care, also showed that cancer patients with VTE had a 1-year survival that was three times lower than that of cancer patients with similar diseases and stages, but who did not have VTE (Noble et al. 2008). And a retrospective review of children with brain tumors and central venous access

dysfunction showed a 2-year survival of only 41 % in those with confirmed thrombotic dysfunction versus 81 % in those without documented central venous access dysfunction (Deitcher et al. 2004).

The mechanisms through which thrombosis contributes to a more aggressive course of malignant disease are not completely understood. However, recent evidence suggests that clinical thrombosis may be more than simply a clinical manifestation of the hypercoagulability of malignancy. Indeed, it appears that thrombosis may provide a local environment that fosters disease progression, with the proteins that participate in thrombus formation and regulation of thrombus extension playing an integral role in stimulating cancer progression by facilitating tumor growth, invasion, angiogenesis, and metastasis (Kakkar and Macbeth 2010).

Thrombin has been shown to induce the expression of monocyte chemotactic protein-1 by monocytes and endothelial cells (Colotta et al. 1994), to stimulate angiogenesis through the release of interleukin-8 and vascular endothelial growth factor (VEGF) (Liu et al. 2006; Chelouch-Lev et al. 2004), and to increase the expression of TF in both endothelial and tumor cells (Zucker et al. 1998). Platelet activation and aggregation may serve as a "nidus" for tumor cells which facilitates tumor cell embolization (Dvorak 1987). Thrombin-induced fibrin network formation facilitates both the binding of tumor cells to the endothelium via ICAM-1 and fibronectin and tumor cell infiltration through the blood vessel wall (Fernandez et al. 2004). Fibrin and platelets also impede the clearing of tumor cells by natural killer (NK) cells (Palumbo et al. 2005). Urokinase enhances tumor cell migration and invasiveness and has both mitogenic and angiogenic effects (Li et al. 1994; Giuliani et al. 1999; Tkachuk et al. 2009). Microvascular thrombosis leads to enhanced expression of TF as well as tumor cell migration away from areas of hypoxia, both of which lead to increased local levels of VEGF, which in turn promotes angiogenesis, microvascular hyperplasia, and rapid tumor expansion (Rong et al. 2005, Sooriakumaran and Kaba 2005; Rudolfsson and Bergh 2009). Aberrant expression of TF has been described in colorectal, pancreatic, breast, and non-oat cell cancers, as well as in melanoma, leukemias, and glioblastoma. Oncogenic events also appear to regulate TF expression and upregulation of the PAI-1 gene (Yu et al. 2005; Boccaccio et al. 2005). In one study, the percentage of tumor cells expressing TF correlated with disease extension in glioma and colorectal cancer (Yu et al. 2005). In patients with advanced (stage IV) and limited (stage I) glioma, the percentage of tumor cells expressing tissue factor was 90 % versus only 20 %, respectively (Yu et al. 2005). In patients with stage IV and stage I colorectal cancer, the percentage of tumor cells expressing tissue factor was 82 % versus only 41 %, respectively (Yu et al. 2005).

Therefore, it is clear that cancer increases the risk of thrombosis, while thrombosis appears to hasten the progress of cancer, and the clinical expressions of such "two-way interaction" lead to a more aggressive course of disease and a negative impact on survival. While the aforementioned mechanisms may not necessarily be present in all patients with cancer, nor in every histological type of malignancy, they do represent important and promising areas for ongoing research and perhaps for development of future targeted therapies.

Treatment of Cancer-Related VTE

Anticoagulant treatment for acute DVT or acute PE typically consists of administration of IV UFH, SC LMWH, or SC fondaparinux followed by transition to an oral VKA. Administration of both IV UFH and oral VKA requires frequent monitoring by clot-based assays. Optimal duration of therapy will depend in part on the etiology of VTE, ranging from 3 months' duration for a situational or provoked VTE to indefinite duration in selected patients with idiopathic or unprovoked VTE (Kearon et al. 2012).

However, the administration and monitoring of anticoagulant therapy in patients with cancer can be very challenging due to a multitude of reasons which are peculiar and more prevalent in patients with malignancies, including the following: (a) true (unfractionated) heparin resistance; (b) difficulties with adequate IV access for IV UFH

Table 11.4 Challenges in the administration and monitoring of anticoagulant therapy in patients with cancer

Unfractionated heparin resistance
Inadequate intravenous access
Frequent blood draws to monitor IV UFH
Underlying coagulopathies
Patients' inability to take oral VKA in the setting of concomitant nausea and vomiting
Anorexia and malnutrition
Malabsorption
Concomitant presence of moderate–severe thrombocytopenia

infusion; (c) difficulties with IV access for frequent blood draws; (d) presence of underlying coagulopathy which may render the activated partial thromboplastin time (aPTT) unreliable to monitor IV UFH therapy, thus requiring the use of anti-Xa activity assays which are not widely available, lack rapid turnaround time for results, and are more costly than aPTT assays; (e) presence of GI side effects from chemotherapy, such as nausea and vomiting, which make it difficult for patients to tolerate oral medicines; (f) occurrence of anorexia and GI side effects that result in dietary changes and variable vitamin K intake, which then can lead to widely fluctuating and erratic INR values; (g) problems with malabsorption or inability to have adequate absorption of oral medicines in patients who underwent extensive bowel resections and have short-gut syndrome and/or require total parenteral nutrition; and (h) uncertainty regarding a "safe" threshold of (chemotherapy-induced) thrombocytopenia above which anticoagulation can still be continued (Table 11.4). Moreover, perhaps the greatest limitation of oral anticoagulant therapy in patients with cancer is the frequent occurrence of true "warfarin failure" and recurrent VTE which can be unacceptably high at times when the INR becomes subtherapeutic, and can be quite frequent even with an adequate INR (Prandoni et al. 2002; Hutten et al. 2000).

These limitations and shortcomings of IV and oral anticoagulation in cancer patients prompted clinical trials evaluating the efficacy and safety of SC LMWH monotherapy compared against oral VKA for the long-term treatment of cancer-associated VTE (Deitcher et al. 2006; Lee et al. 2003; Meyer et al. 2002; Hull et al. 2006). In these prospective, open-label, randomized controlled trials, the experimental groups were treated with weight-based LMWH monotherapy for 3–6 months, while control groups were given anticoagulation with an oral VKA such as warfarin (adjusted to a target INR of 2.0–3.0). In all but one of those trials, the primary efficacy outcome was symptomatic recurrent VTE and the primary safety outcome was major bleeding during the observation period.

In the CLOT trial ($n=672$) (Lee et al. 2003), patients were randomized to dalteparin versus warfarin for 6 months. The dose regimen for dalteparin was 200 IU/kg SC for 1 month, followed by 150 IU/kg SC for the remainder 5 months, whereas the control group was treated with dalteparin 200 IU/kg for 5–7 days, followed by warfarin (adjusted to a target INR 2.0–3.0) for 6 months. Rates of recurrent VTE were 17 and 9 % in the warfarin and dalteparin monotherapy groups, respectively. Rates of major bleeding were 4 and 6 % in the warfarin and dalteparin monotherapy groups, respectively. Dalteparin monotherapy for 6 months resulted in a 52 % relative risk reduction in recurrent VTE events (hazard ratio**,** HR, 0.48; $p<0.002$) (Lee et al. 2003).

In the CANTHANOX trial ($n=138$) (Meyer et al. 2002), patients were randomized to enoxaparin versus warfarin for 3 months. The dose regimen for enoxaparin was 1.5 mg/kg SC once daily, whereas the control group received enoxaparin 1.5 mg/kg SC once daily for at least 4 days, followed by warfarin (adjusted to a target INR of 2.0–3.0). Rates of the composite endpoint of recurrent VTE and major bleeding were 21.1 and 10.5 % in the warfarin and enoxaparin monotherapy groups, respectively ($p=0.09$) (Meyer et al. 2002).

In the LITE trial ($n=200$) (Hull et al. 2006), patients were randomized to tinzaparin versus warfarin for 3 months. The dose regimen of tinzaparin was 175 IU/kg SC once daily, whereas the control group was treated with IV UFH for a minimum of 6 days, followed by warfarin (adjusted to a target INR of 2.0–3.0). Rates of the recurrent VTE were not statistically different between the

Table 11.5 Rates of recurrent VTE in prospective randomized trials comparing LMWH and warfarin

	Rate of recurrent VTE (%)	
Study	LMWH	Warfarin
CLOT (dalteparin, $n=672$)	9	17
LITE (tinzaparin, $n=200$)	6	10
CANTHANOX (enoxaparin, $n=138$)	3	4
ONCENOX (enoxaparin, $n=123$)	6.6	10

Table 11.6 Advantages of LMWH over oral VKA and unfractionated heparin for treatment of cancer-related VTE

Lack of need for laboratory monitoring
No significant drug–drug interactions with chemotherapy agents
No drug–diet interactions
No variability in plasma levels of LMWH regardless of patients' nutritional status
Shorter plasma half-life facilitates short-term interruptions prior to invasive procedures
Lower rates of heparin-induced thrombocytopenia compared to UFH

two groups: 10 and 6 % in the warfarin and tinzaparin groups, respectively. Major bleeding rates were 7 % in both groups (Hull et al. 2006).

In the ONCENOX trial ($n=101$) (Deitcher et al. 2006), patients were randomized into three treatment arms: group 1a ($n=29$) was treated with enoxaparin 1.0 mg/kg SC twice daily for 5 days, followed by enoxaparin 1.0 mg/kg SC once daily for 175 days; group 1b ($n=32$) was treated with enoxaparin 1.0 mg/kg SC twice daily for 5 days, followed by enoxaparin 1.5 mg/kg SC once daily for 175 days; and group 2 ($n=30$) was treated with enoxaparin 1.0 mg/kg SC twice daily for a minimum of 5 days and until an INR target of 2.0–3.0 was achieved on warfarin (which was started on day#2 of enoxaparin therapy). In the intention-to-treat analysis ($n=91$), recurrent VTE occurred in 6.9 % of patients in group 1a, 6.3 % of patients in group 1b, and 10 % of patients in group 2. Because of the small number of VTE events, differences between groups were not statistically significant (Table 11.5) (Deitcher et al. 2006).

A recent Cochrane meta-analysis of all clinical studies of anticoagulation with an LMWH for the long-term treatment of VTE in patients with cancer revealed a 53 % relative risk reduction in recurrent VTE for those treated with LMWH as opposed to oral VKA (HR, 0.47; 95 % CI, 0.32–0.71), but no statistically significant differences in major hemorrhage (Akl et al. 2011a, b June 15). Several guidelines have recommended LMWH over oral VKA as the anticoagulant regimen of choice in patients with cancer who develop acute VTE (Kearon et al. 2012; Mandalà et al. 2011; Lyman et al. 2007).

There are other practical advantages of SC LMWH over IV UFH and oral VKA for initial and long-term VTE treatment in patients with cancer that go beyond efficacy in preventing recurrent VTE and include (a) lack of need for monitoring due to predictable pharmacokinetics and pharmacodynamics when administered as weight-based dosing; (b) no significant drug–drug interactions with chemotherapy agents or other drugs; (c) no need to adjust dosing or interrupt therapy in case of fluctuations or variability in patients' nutritional status, diet, vitamin K intake, and GI side effects; (d) convenience of shorter plasma half-life which makes it easier to interrupt and resume therapy around times of surgeries or invasive procedures; and (e) lower rates of heparin-induced thrombocytopenia (HIT) compared to UFH (Table 11.6).

However, the single major disadvantage of LMWH when compared with IV UFH and warfarin is that LMWHs should not be used in patients with renal insufficiency (defined as a calculated creatinine clearance <30 mL/min) and are absolutely contraindicated in patients with end stage renal disease. Disadvantages of LMWH compared to oral VKA include (a) the fact that, in many countries, the cost of LMWH is prohibitive and not affordable for patients without medical insurance and (b) the need for daily SC injections which may negatively impact compliance with treatment, although some studies have shown improvement in quality of life compared to other antithrombotic drugs (Lee et al. 2003; Noble and Finlay 2005).

The exquisite hypercoagulability of malignancy can also be illustrated by the fact that recurrent

VTE can also occur despite ongoing LMWH therapy. Reported recurrent VTE rates in patients treated with LMWH monotherapy range from 6 to 10 % (Deitcher et al. 2006; Lee et al. 2003; Meyer et al. 2002; Hull et al. 2006; Monreal et al. 2004), with one study reporting no VTE recurrences (Noble et al. 2007). While VTE recurrences may be explained by poor compliance with injections in some patients, available evidence suggests that an interval of less than 3 months between VTE and cancer diagnosis, younger age, presence of metastases, and an Eastern Cooperative Oncology Group (ECOG) performance status of two before acute VTE are predictors of VTE recurrence while on anticoagulation (Deitcher et al. 2006; Trujillo-Santos et al. 2008, Louzada et al. 2011; Lee 2010; Lee et al. 2009). In addition, a subgroup analysis of the Enoxaparin Clinical Trial Group (529 study), which was a randomized prospective controlled trial comparing two different enoxaparin dosing regimens for treatment of acute VTE (1.0 mg/kg SC twice daily vs. 1.5 mg/kg SC once daily), found that the once-daily enoxaparin regimen was associated with higher rates of recurrent VTE in cancer patients (6 of 49 patients; 12.2 %) compared to the twice-daily regimen (3 of 47 patients; 6.4 %) (Merli et al. 2001). Thus, the once-daily regimen of enoxaparin may not represent optimal dosing for some patients with active malignancy.

While best available evidence suggests that LMWH monotherapy for 3–6 months is the preferred method of anticoagulation in patients with cancer-associated VTE, the optimal duration of long-term therapy (beyond the first 3–6 months) is unknown. As a general rule, most experts agree that anticoagulant therapy should be continued for as long as a cancer patient has clinical evidence of active disease and for as long as there are persistent risk factors, such as ongoing chemotherapy. In addition, indefinite therapy is typically recommended for patients with known metastases (Kearon et al. 2012; Mandalà et al. 2011). The ideal anticoagulant regimen beyond the first 3–6 months is also undefined because of lack of prospective controlled data. Guidelines typically recommend either oral VKA or LMWH monotherapy based on patient-specific circumstances and preferences (Kearon et al. 2012).

Primary Prophylaxis Against VTE in Cancer Patients

Prevention of Postoperative VTE

Because cancer patients undergoing surgery have such a high risk of postoperative VTE, current guidelines recommend pharmacological prophylaxis for at least 7–10 days in patients undergoing surgery for cancer (Gould et al. 2012; Mandalà et al. 2011; Lyman et al. 2007, 2013). Such prophylaxis is typically administered in the form of SC UFH 5,000 IU every 8 h or SC LMWH once daily. Fondaparinux has been found to be at least as effective as dalteparin for prevention of postoperative VTE in patients undergoing high-risk abdominal surgery in one prospective randomized trial (Agnelli et al. 2005). In a post hoc analysis of the 68 % of study patients who had cancer and were included in that trial, fondaparinux was associated with a 39 % relative risk reduction in postoperative VTE compared to dalteparin (Agnelli et al. 2005), but fondaparinux is not FDA-approved for this indication.

In addition, because the hypercoagulability of malignancy increases cancer patients' risk of postoperative VTE for at least 4 weeks following major surgery, clinical trials were conducted addressing the efficacy and safety of extended postoperative pharmacological prophylaxis in this patient population. The ENOXACAN II trial ($n=332$ in the intention-to-treat analysis of efficacy) randomized patients undergoing abdominal surgery for cancer to receive either enoxaparin 40 mg SC once daily or placebo for another 21 days, following a standard 6- to 10-day administration of enoxaparin 40 mg SC once daily (Bergqvist et al. 2002). Rates of all VTE events (including any acute lower-extremity DVT and acute PE) were 12 and 4.8 % at the end of the 4-week double-blind phase and 13.8 and 5.5 % at the end of 3 months of follow-up, in the placebo and enoxaparin groups, respectively. This corresponded to a statistically significant 60 % relative risk reduction of VTE in favor of extended prophylaxis with enoxaparin without any significant differences in major bleeding (Bergqvist et al. 2002). The CANBESURE study ($n=488$ in the

intention-to-treat efficacy analysis) randomized patients undergoing abdominal or pelvic surgery for cancer to receive either bemiparin 3,500 IU SC once daily or placebo for another 20 days, following a standard 8-day administration of bemiparin 3,500 IU SC once daily (Kakkar et al. 2010). Rates of all VTE events (all DVT and PE) were 13.3 and 10.1 % in the placebo and bemiparin groups, respectively, for a 24 % relative risk reduction of VTE in favor of bemiparin without any significant differences in major bleeding (Kakkar et al. 2010).

A Cochrane review of extended LMWH prophylaxis after abdominal or pelvic surgery showed that extended prophylaxis is associated with 75 % reduction in above-the-knee acute DVT events, as well as greater than 50 % reduction in any acute lower-extremity DVT events (including calf DVT) compared to prophylaxis administered for 7–10 days (Rasmussen et al. 2009). At least two current guidelines recommend extended-duration pharmacological prophylaxis with LMWH for 4 weeks over prophylaxis for 1 week in cancer patients undergoing abdominal or pelvic surgery (Gould et al. 2012; Mandalà et al. 2011).

Prevention of Catheter-Related Upper-Extremity VTE

Despite evidence that select patients with cancer and central venous catheters may benefit from prophylactic-intensity heparins or low-dose warfarin (Akl et al. 2011a, b; Young et al. 2009), current guidelines either do not recommend (Mandalà et al. 2011; Lyman et al. 2007) or recommend against primary prevention against VTE in cancer patients with central venous catheters (Gould et al. 2012; Mandalà et al. 2011).

Prevention of Chemotherapy-Related VTE

There have been four prospective trials evaluating the safety and efficacy of primary thromboprophylaxis in cancer patients undergoing chemotherapy (Agnelli et al. 2009; Haas et al. 2012; Maraveyas et al. 2012; Agnelli et al. 2012, 2013a). The PROTECHT trial randomized ambulatory patients receiving chemotherapy for advanced lung, GI, pancreatic, breast, ovarian, or head and neck cancer to receive nadroparin 3,800 IU SC once daily ($n=779$) or placebo ($n=387$) for up to 4 months (Agnelli et al. 2009). The study showed a nearly 50 % relative risk reduction in symptomatic thromboembolic events—2.0 % versus 3.9 % in the nadroparin and placebo groups, respectively ($p=0.02$). There were no statistically significant differences in the rates of major or minor bleeding complications between the two groups (Agnelli et al. 2009).

The TOPIC trials, evaluating primary thromboprophylaxis with certoparin—3,000 IU SC once daily—in patients with advanced breast (TOPIC-1) or non-small cell lung cancer, NSCLC (TOPIC-2), failed to show a similar benefit of thromboprophylaxis. Nevertheless, a nonstatistically significant trend towards benefit was observed in patients with NSCLC, with VTE rates of 4.5 and 8.3 % seen in patients treated with certoparin and placebo, respectively ($p=0.07$) (Haas et al. 2012). This latter finding prompted a combined analysis of the PROTECHT and TOPIC-2 trials, in order to evaluate the clinical benefit of LMWH prophylaxis in patients with metastatic or locally advanced lung cancer receiving chemotherapy (Verso et al. 2010). A total of 811 patients were included in this analysis, which showed a 42 % relative risk reduction in symptomatic VTE events (3.2 and 5.5 % in patients treated with LMWH and placebo, respectively) and a 46 % relative risk reduction in all—both symptomatic and asymptomatic—VTE events (4.3 and 7.8 % in patients treated with LMWH and placebo, respectively) (Verso et al. 2010).

The clinical benefit of therapeutic-intensity doses of SC dalteparin was evaluated in a prospective trial which included 123 patients with advanced pancreatic cancer receiving gemcitabine. Patients were randomized to gemcitabine 1,000 mg/m^2 with or without dalteparin administered in a weight-adjusted regimen similar to the one used in the CLOT trial: 200 IU/kg for the first month, followed by 80 % of the initial dose for 12 weeks (Maraveyas et al. 2012). The primary outcome was the incidence of all arterial and

venous thromboembolic events at 12 weeks ("treatment period") and at 100 days ("follow-up period," when many patients underwent imaging studies for restaging of disease). At 12 weeks, there was an 85 % relative risk reduction in the incidence of thromboembolism in the group of patients receiving gemcitabine + dalteparin (3.4 %) compared to the group receiving gemcitabine alone (23 %), for a $p = 0.002$. At the end of the "follow-up period," after all incidental VTE events found during restaging were included, the relative risk reduction was 58 % (12 % vs. 28 %, $p = 0.039$, in the gemcitabine + dalteparin vs. gemcitabine alone group, respectively). Despite the fact that therapeutic-intensity doses of dalteparin were used in patients without a diagnosis of acute thrombosis, major bleeding rates were identical in both groups (3.2 and 3.4 % in the gemcitabine and gemcitabine + dalteparin groups, respectively). Rates of minor bleeding (particularly minor epistaxis and skin bruising) were higher in the LMWH-treated group (Maraveyas et al. 2012).

The more recent SAVE-ONCO trial was a multicenter, randomized, double-blind, placebo-controlled trial evaluating the efficacy and safety of semuloparin (an ultralow-molecular-weight heparin) at a dose of 20 mg SC once daily compared to placebo (Agnelli et al. 2012). This study ($n = 3{,}212$) included patients with metastatic (68 %) or locally advanced cancer of the lungs (37 %), colon–rectum (29 %), stomach, ovary, pancreas, and bladder who were beginning to receive a new course of chemotherapy. Semuloparin was administered until there was a change of chemotherapy regimen (median treatment duration of 3.5 months). Rates of the composite primary efficacy outcome (any symptomatic DVT, nonfatal PE, and VTE-related death) were 1.2 and 3.4 % in the groups treated with semuloparin and placebo, respectively (HR, 0.36; 95 % CI, 0.21–0.60; $p < 0.001$). This 64 % relative risk reduction in the primary endpoint was observed without a statistically significant increase in major bleeding complications (Agnelli et al. 2012).

Despite the aforementioned evidence, current major guidelines do not recommend routine VTE prophylaxis in ambulatory cancer patients who undergo chemotherapy (Mandalà et al. 2011; Lyman et al. 2007, 2013; Kahn et al. 2012). The most recent guidelines by the American College of Chest Physicians (ACCP) specifically recommend against routine prophylaxis with UFH or LMWH in ambulatory patients with cancer who have no additional risk factors for VTE (Grade 2B recommendation) (Kahn et al. 2012). The ACCP guidelines list a history of previous venous thrombosis, immobilization, hormonal therapy, angiogenesis inhibitors, thalidomide, and lenalidomide as additional risk factors for VTE in this patient population. The same guidelines further "suggest" (Grade 2B recommendation) prophylactic LMWH or UFH over no prophylaxis in ambulatory patients with solid tumors who have one of those additional risk factors for VTE (Kahn et al. 2012). The most recent guidelines by the American Society of Clinical Oncology (ASCO) further state that, based on limited data, LMWH prophylaxis may be considered on a case-by-case basis in highly selected outpatients with solid tumors and that patients with multiple myeloma receiving thalidomide- or lenalidomide-based regimens should receive prophylaxis with either aspirin or LMWH for lower-risk patients and LMWH for higher-risk patients (Lyman et al. 2013).

Anticoagulants and Cancer Survival

The earliest observation of a possible benefit of antithrombotic use in cancer survival dates back to 1954 (Albert-Weil and Nehorias 1954). Since then, a few studies have attempted to determine whether warfarin, UFH, or LMWH would lead to improved survival in cancer patients (Zacharski et al. 1984; Chahinian et al. 1989; Maurer et al. 1997; Lebeau et al. 1994; Kakkar et al. 2004; Klerk et al. 2005; Altinbas et al. 2004).

Although warfarin use was associated with improved survival in a small subset of patients with NSCLC (Zacharski et al. 1984), those findings were not corroborated by two other studies (Chahinian et al. 1989; Maurer et al. 1997). The use of chemotherapy combined with dose-adjusted SC UFH for 5 weeks in 277 patients with SCLC was associated with improved median survival compared to chemotherapy alone (317 days vs. 261 days; $p = 0.01$) (Lebeau et al. 1994).

Mounting evidence that coagulation proteins involved in thrombus formation also play a role in cancer cell growth and dissemination has renewed interest in the possible antitumoral effect of anticoagulants in general and LMWH in particular (Hettiarachchi et al. 1999). Indeed, meta-analyses of clinical trials comparing LMWH and UFH for VTE treatment suggested that LMWH was associated with lower mortality in the subgroup of patients with cancer (Lensing et al. 1995; Siragusa et al. 1996). More recently, a positive effect of LMWH in improving survival in cancer patients was suggested or demonstrated in three prospective, randomized trials (Kakkar et al. 2004; Klerk et al. 2005; Altinbas et al. 2004).

The FAMOUS trial randomized 385 patients with advanced breast, lung, GI, pancreatic, hepatic, GU, ovarian, or uterine cancer to usual chemotherapy with and without prophylactic-intensity dalteparin, 5,000 IU SC once daily. The primary endpoint (1-year mortality) was identical in both groups (Kakkar et al. 2004). A post hoc analysis of patients who survived more than 17 months demonstrated a survival benefit of dalteparin, with 2-year and 3-year survival rates of 78 % versus 55 % and 60 % versus 36 % in the dalteparin versus placebo groups, respectively (Kakkar et al. 2004).

Another trial prospectively evaluated the use of nadroparin in 302 patients with metastatic or locally advanced cancer who could not be treated with curative intent (Klerk et al. 2005). Patients were randomized to a 6-week course of weight-adjusted (therapeutic-intensity) nadroparin (twice daily for the first 2 weeks, once daily for the remainder 4 weeks) or placebo, in addition to their usual chemotherapy regimen(s). Although survival at 6 months did not differ between the nadroparin and placebo groups (61 and 56 %, respectively), those rates were 39 and 27 % at 12 months and 21 and 11 % at 24 months for the nadroparin and placebo groups, respectively. Overall, the median survival effect of nadroparin was significantly increased compared to placebo: 8.0 versus 6.6 months, respectively (HR = 0.75; 95 % CI, 0.59–0.96; $p = 0.021$) (Klerk et al. 2005).

The effect of LMWH on survival was also studied prospectively in patients with SCLC (Altinbas et al. 2004). In this study, a total of 84 patients were randomized to receive chemotherapy alone or chemotherapy plus prophylactic-intensity dalteparin (5,000 IU SC once daily) for 18 weeks. Median overall survival was 13 months and 8 months in the chemotherapy + dalteparin and chemotherapy alone groups, respectively. This improvement in survival was observed in patients with both limited and extensive disease stages. Overall tumor response rates were 69.2 and 42.5 % in the chemotherapy + dalteparin and chemotherapy alone groups, respectively (Altinbas et al. 2004).

Two of the abovementioned prospective trials shared the common observation that the survival benefit associated with LMWH was most apparent among patients who had better prognosis at the time of enrollment (i.e., those estimated to live 6 months or longer) (Kakkar et al. 2004; Klerk et al. 2005). However, a phase-III clinical trial ($n = 141$) did not observe survival benefit of LMWH over saline injections (Sideras et al. 2006). This study was originally double-blind but became an open-label trial due to poor enrollment (Sideras et al. 2006).

A meta-analysis of the four trials discussed above concluded that use of LMWH does improve overall survival in cancer patients, with a pooled hazard ratio of 0.83 (95 % CI, 0.70–0.99; $p = 0.03$) in all patients with cancer (Lazo-Langner et al. 2007). Contrary to previous observations from studies employing UFH (which suggested a survival benefit only in patients with limited disease) (Lebeau et al. 1994), the survival benefit associated with LMWH is apparent even in those with advanced disease (HR = 0.86; 95 % CI, 0.74–0.99; $p = 0.04$) (Lazo-Langner et al. 2007). Despite these encouraging data, routine use of LMWH with the specific intent of cancer survival, in patients without VTE, is not currently recommended.

Future Perspectives

Prediction of VTE in Cancer Patients

The frequency of VTE, the negative impact of VTE on disease progression and patient survival, as well as the challenges and complications associated

with anticoagulant therapy in patients with cancer represent legitimate reasons to pursue an aggressive preventive approach against VTE in this patient population. However, despite the fact that the use of prophylactic LMWH in selected outpatients with cancer appears to reduce the risk of VTE with minimal side effects, identification of patients who may benefit the most from primary prophylaxis remains very difficult because the risk of VTE varies according to different histological types, disease stages, and chemotherapy protocols. Moreover, the ideal anticoagulant regimen and treatment duration are also unknown.

Strategies that have been published or are under investigation to assist in the identification of high-risk cancer patients who could potentially be ideal candidates for primary VTE prophylaxis include clinical risk scoring models and the use of biomarkers (Khorana 2012; Khorana et al. 2008; Ay et al. 2010). P-selectin is a cell-adhesion molecule expressed by activated platelets and endothelial cells; it participates in coagulation pathways that lead to thrombin generation, and it also appears to facilitate interactions between cancer cells and the endothelium. Elevated sP-selectin was associated with a 2.6-fold increased risk of VTE in a cohort of 687 patients with cancer, including hematologic malignancies (actual VTE rates were 11.9 % vs. 3.7 % in patients with elevated soluble P-selectin (sP-selectin) compared to those with lower levels, respectively) (Ay et al. 2008). Elevated levels of TF-bearing microparticles (TFMP) measured by impedance-based flow cytometry have been associated with a fourfold increased risk of VTE in cancer outpatients in a case-control study (Zwicker et al. 2009). More recently, a randomized, phase-II trial of primary thromboprophylaxis with enoxaparin in cancer patients with elevated TFMP was completed (Zwicker et al. 2013). The study included patients with stage IV colon cancer, NSCLC (stage IIIb or IV), or pancreatic cancer (stage III or IV) who were within 4 weeks of initiating 1st- or 2nd-line chemotherapy ($n = 70$). Patients with elevated TFMP were randomized to enoxaparin 40 mg SC once daily for 60 days versus observation. Those with low levels of TFMP were also observed during the study period, and both observation groups were followed in a double-blind manner with respect to TFMP status. The cumulative incidence of VTE at 2 months in the higher TFMP group randomized to observation was 27.3 % versus 5.6 % in the group randomized to enoxaparin. The VTE rate in the enoxaparin group was similar to that of the group with low TFMP levels (7.2 %) (Zwicker et al. 2013).

While the use of biomarkers is a promising tool to predict VTE risk and potentially guide therapy decisions, laboratory assays to measure many biomarkers, including sP-selectin and TFMP, are neither standardized nor widely available for clinical use to this date. Moreover, phase-III trials are required to determine whether therapy interventions based on a given biomarker level will meaningfully impact clinical outcomes.

New Oral Anticoagulants for Cancer-Associated VTE

Novel oral anticoagulants that do not require laboratory monitoring and, unlike LMWH, do not require daily SC injections have become potentially valid alternatives to oral warfarin and LMWH for patients with VTE. However, to date there are no prospective clinical trials specifically addressing the efficacy and safety of new oral anticoagulants in the treatment of cancer-associated VTE. In addition, only a minority of patients included in the prospective clinical trials of new oral anticoagulants for VTE treatment had active cancer at the time of inclusion: 1.8, 2.5, 6.8, and 5 % in the AMPLIFY-EXT (apixaban), AMPLIFY (apixaban), RE-COVER (dabigatran), and EINSTEIN-DVT (rivaroxaban) trials, respectively (Agnelli et al. 2013a, b; Schulman et al. 2009; The EINSTEIN Investigators 2010).

Thus, current data of safety and efficacy cannot be extrapolated to patients with cancer-related VTE. This is especially true in patients with chemotherapy-induced thrombocytopenia, in whom the risk of major hemorrhage associated with new oral anticoagulants is simply unknown at this time. As with oral warfarin, consistent use of new oral anticoagulants may also be very

difficult in patients with significant nausea, vomiting, or malabsorption. In addition, because the metabolism of novel oral anticoagulants is dependent on the p-glycoprotein transporter or CYP3A4 pathways, concomitant use of inhibitors or inducers of those pathways will lead to increase or decrease in the plasma levels of anticoagulants. Chemotherapy drugs that interfere with those pathways include dexamethasone, doxorubicin, vinblastine, cyclosporine, lapatinib, nilotinib, sunitinib, tamoxifen, and imatinib (Lee 2012). These potentially significant drug interactions may indeed become a contraindication to the use of new oral anticoagulants in patients who require the use of such chemotherapy agents. Therefore, while new anticoagulants may eventually play an important role in the management of VTE in cancer patients, dedicated prospective studies in cancer-related VTE are required. The most recent American Society of Clinical Oncology (ASCO) Practice Guidelines Update on VTE prophylaxis and treatment in patients with cancer does not recommend new oral anticoagulants for treatment of patients with malignancy-related VTE.

Clinical Vignette: Conclusion

Within 6 months since discontinuation of *cis-retinoic* acid and warfarin therapies, he developed new-onset headaches. Brain MRI revealed a new enhancing lesion at the left temporal resection site. He declined surgery and was started on bevacizumab. Three months later, he had a syncope event and was diagnosed with acute PE, as well as a new right lower-extremity acute popliteal and calf DVT. He was treated with IV UFH initially, and within a few days his therapy was switched to enoxaparin 1.5 mg/kg SC once daily. He remains on chemotherapy with bevacizumab and on anticoagulation therapy with once-daily enoxaparin injections to this date.

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Thrombotic Risk Factors

12

Erika Leemann Price and Tracy Minichiello

Clinical Vignette 1

A 68-year-old man with past medical history notable only for hypertension and well-controlled diabetes mellitus presents with right lower leg swelling and pain. He denies recent prolonged travel, surgery, or trauma. He denies shortness of breath or chest pain. Ultrasound reveals occlusive thrombus extending from the popliteal vein proximally into the common femoral vein.

Introduction

Incidence of venous thromboembolism (VTE), including deep venous thrombosis (DVT) and pulmonary embolism (PE), is around 100 cases per 100,000 population, based on studies in predominantly Caucasian populations, with slightly lower risks in registries focused on Hispanic, African Americans, and Asian populations (White 2003). Incidence rises with increasing age (White 2003; Naess et al. 2007).

Pathophysiologic understanding of VTE is largely credited to Rudolph Virchow, whose mid-nineteenth century writings challenged the prevailing concept that pulmonary emboli originated in situ and demonstrated their origins in the peripheral venous system. His description of the consequences of pulmonary emboli included "phenomena due to the irritation of the vessel and its surroundings…phenomena due to blood coagulation…[and] phenomena due to the interruption of the bloodstream" (Virchow 1998; Kumar et al. 2010). Through his and others' work, "Virchow's Triad" of wall stress, hypercoagulability, and stasis later became known as classic risk factors for VTE. Subsequent work has deepened and broadened the mechanistic understanding of VTE, including emphasis on the roles of low oxygen tension in stasis, activation of the endothelium, activation of innate and acquired immune systems, platelet activation, and levels of pro- and anticoagulant proteins (Reitsma et al. 2012). Understanding of the contributions of these factors to VTE risk in individuals and in particular disease states continues to evolve.

Here we present a summary of VTE risk factors for the clinician, stressing the relative importance and clinical impact of each. We begin with the factors that confer highest VTE risk and move towards those that are less significant. In presenting incidence and relative risk, we focus primarily on *symptomatic* VTE; incidence of asymptomatic VTE may be much higher but has less clinical relevance. Available data are complicated by methodological differences in detection of events and differing thresholds for considering VTE "symptomatic."

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Although certain risk factors may be listed as "minor," it is important to note that even minor risk factors are additive and multiple minor risk factors together can create substantial risk for VTE. This is particularly important when risk factors have multiplicative interactions, as with Factor V Leiden or prothrombin G20210A heterozygosity and use of combined oral contraceptives.

Approach to the Newly Diagnosed DVT or PE

A critical step in evaluation of a patient with a first VTE event is determination of whether the event is provoked or unprovoked. This classification helps determine risk of recurrence if anticoagulation is stopped and therefore informs duration of therapy. The definition of "unprovoked" varies as used in the literature, but generally refers to VTE in the absence of a transient risk factor such as recent trauma or major surgery. Patients with unprovoked VTE have higher recurrence risk than those with provoked VTE. It is this determination that will most strongly influence recommendations around duration of therapy, and therefore, significant effort should be made to identify contributing risk factors. In the setting of average risk of bleeding, patients with even a single unprovoked VTE are now considered candidates for indefinite anticoagulation, while those with VTE related to transient risk factors receive a minimum of 3 months of therapy (Kearon et al. 2012).

For up to 75 % of patients with VTE, at least one risk factor can be identified (White 2003). Routine testing of all patients with VTE for laboratory thrombophilia is discouraged, as such testing is unlikely to change the course of management in many cases. However, the following questions may help determine whether further evaluation is likely to be helpful for an individual patient:

- Will further evaluation change duration of therapy? A determination of a major inherited or acquired thrombophilia may warrant indefinite anticoagulation. For otherwise healthy younger patients with apparently unprovoked VTE, suspicion for thrombophilia may be higher and lead to further evaluation. A strong family history or an event that occurs after only mild provocation may increase suspicion for an underlying hypercoagulable state.
- Is the patient considering pregnancy? Laboratory thrombophilia may have important prognostic significance with regard to pregnancy complications and outcomes, and identification may result in recommendations for anticoagulation or antiplatelet therapy in the antepartum and peripartum period.

A search for malignancy is indicated in patients presenting with unprovoked VTE who are 50 years of age or older and in those with recurrent VTE despite anticoagulation. Cancer-related VTE calls for ongoing treatment with low-molecular-weight heparin instead of transition to warfarin.

The patient in Clinical Vignette 1 has had an unprovoked first VTE at age 68. As this was an unprovoked, proximal DVT, the patient would be considered for indefinite anticoagulation in the absence of contraindications, and further evaluation for thrombophilia is unlikely to change this recommendation. However, given his age, occult malignancy is a concern and would change the type of anticoagulation recommended. Further evaluation should focus on determination of additional historical risk factors for VTE and/or malignancy (such as tobacco use) and age-appropriate malignancy screening. Review of laboratory studies including complete blood count, electrolytes, and renal and hepatic function may also suggest abnormalities warranting further evaluation.

Major VTE Risk Factors

Clinical Vignette 2

An 80-year-old woman is admitted for hip fracture following a mechanical fall; 3 days later she is taken to the operating room for an open reduction and fixation. VTE prophylaxis with low-molecular-weight heparin is initiated on hospital day 4 (postoperative day 1). On hospital day 5 (postoperative day 2), she develops acute shortness of breath; a CT of the chest with contrast reveals bilateral segmental pulmonary emboli.

Major Orthopedic Surgery: Hip Fracture, Hip Repair, Knee Repair

Major surgery and trauma confer strong risks for VTE (Table 12.1). Arthroplastic orthopedic surgery of the hip or knee carries especially high VTE risk; up to 50 % of patients undergoing total knee or total hip replacements develop DVT and/or PE, although only 5 % or fewer are symptomatic (Anderson and Spencer 2003; Falck-Ytter et al. 2012). Improvements in surgical technique over the past decade leading to less thrombogenic protocols and routine use of prophylaxis have reduced postoperative VTE risk considerably. Limited data suggest that the risk of VTE following hip arthroplasty is greater than that for arthroplastic knee surgery (Falck-Ytter et al. 2012). Risk is increased with application of a tourniquet for over 60 min (Bergqvist and Lowe 2002).

VTE risk is highest immediately after surgery but remains elevated for several weeks. Approximately two thirds of VTE events detected in the 35-day postoperative period occur within the first 2 weeks, but up to 70 % of VTE events are not detected until after hospital discharge, and risk may not return to baseline until approximately 3 months after surgery. This period may be somewhat shorter in patients undergoing knee arthroplasty than in patients undergoing hip arthroplasty (Falck-Ytter et al. 2012; White et al. 1998; Bjornara et al. 2006). Duration of risk varies individually with degree of persistent immobility and presence of additional risk factors.

Table 12.1 Risk factors for venous thromboembolism

Major (odds ratio 10 or higher)	Moderate (odds ratio 2–9)	Minor (odds ratio 2 or lower)
Fracture (hip or leg)	Arthroscopic knee surgery	Laparoscopic surgery
Hip or knee replacement	Central venous lines	Bed rest (at least 3 days)
Major general surgery	Chemotherapy	Travel >4 h
Major trauma or spinal cord injury	Oral contraceptives	Increasing age
	Hormone replacement therapy	Obesity
	Malignancy	Pregnancy (antepartum)
	Paralytic stroke	Varicose veins
	Pregnancy (postpartum)	Tobacco use
	Previous venous thromboembolism	Dyslipidemia
	Thrombophilia	Poorly controlled diabetes
	Autoimmune disease	Chronic renal disease
	Nephrotic syndrome	
	Congestive heart failure	
	Respiratory failure	

Adapted with permission from Anderson FA, Jr., Spencer FA. Risk factors for venous thromboembolism. Circulation. 2003;107(23 Suppl 1):I9–16

Major General Surgery

While minor or outpatient procedures confer minimal DVT risk, the risk increases with major general surgery, generally defined as abdominal or thoracic surgeries involving at least 30 minutes of anesthesia (Anderson and Spencer 2003; Gould et al. 2012). Open gynecologic and urologic surgeries, as well as invasive neurosurgery, also carry particularly elevated VTE risk (Gould et al. 2012; White 2003). Patients undergoing open abdominal or pelvic surgery for cancer appear to be at highest risk, while less invasive operations such as inguinal hernia repair and mastectomy confer lower risk (Gould et al. 2012). Patients undergoing laparoscopy may have less tissue trauma, be mobilized earlier, and have shorter hospital stays than those with open surgeries, leading to lower risk for VTE (Bergqvist and Lowe 2002).

Presence of additional risk factors including cancer, advanced age, medical comorbidities, and complications of surgery contributes to VTE risk in surgical patients (Gould et al. 2012).

Trauma

VTE is a common complication of major trauma. In general, patients with higher severity of injuries are at elevated risk for DVT and PE; a prospective study of trauma patients admitted with an injury severity score of at least 9 found DVT in 58 %, although very few of these were symptomatic (Geerts et al. 1994).

In addition to general severity of trauma, specific types of trauma appear to pose additional risk, including fractures in the lower extremities, vascular injury, and spinal cord injury (Gould et al. 2012; Geerts et al. 1994). Studies from the early 1990s indicate an incidence of DVT (symptomatic and asymptomatic) approaching 40 % over 3 months following injury, with a 5 % incidence of PE (Anderson and Spencer 2003). Risk of VTE following spinal cord injury is highest immediately following the event and over the 2 weeks following injury, but persists throughout the rehabilitation period (Gould et al. 2012).

Prolonged immobilization and surgery compound the risk of VTE in trauma patients. However, the risk posed by immobility alone is much lower, as discussed below; thus other mechanisms related to the effect of trauma and inflammation on hypercoagulability are also likely to be at play.

The patient in Clinical Vignette 2 is at high risk for VTE based on her age and the type of injury she has sustained compounded by her immobility preoperatively while in hospital awaiting her procedure. Attention should be paid to VTE risk factors both pre- and postoperatively in these patients, and prophylaxis should be administered to those not proceeding immediately for surgical repair (Falck-Ytter et al. 2012).

Moderate Risk Factors

Major Inherited and Acquired Thrombophilias

The major inherited and acquired thrombophilias are antiphospholipid syndrome; deficiencies of protein C, protein S, or antithrombin; and hyperhomocysteinemia. Inherited genetic mutations including Factor V Leiden and the prothrombin G20201A gene mutation, though common, do not alone confer high VTE risk. However, they gain importance in conjunction with additional risk factors particularly in the homozygous form or in combination with each other (heterozygous Factor V Leiden and heterozygous prothrombin gene mutation).

Extensive testing for thrombophilia, though often performed, may not be helpful in patients for whom indefinite anticoagulation is indicated regardless of test result. Onset of VTE in older patients is much more likely to be associated with malignancy than with an acquired or inherited thrombophilia; therefore, evaluation in these patients should focus on age-appropriate cancer screening and a careful review of patient history

and recent laboratory work to elicit data that may suggest need for additional testing. For younger patients with unexplained VTE and higher probability of thrombophilia, testing may be warranted to help with understanding of risks and benefits associated with discontinuing anticoagulation. Testing may also have additional benefit in patients with strong family history for VTE for whom other family members may be affected by results. For women of childbearing age who present with VTE, testing carries particular importance given the need for planning around potential pregnancies.

Some patients may present a clinical picture suggestive of a major thrombophilia, and in these cases testing may help to confirm the diagnosis and plan treatment. For instance, a younger patient with arterial and venous thrombosis and/or recurrent pregnancy loss should be evaluated for antiphospholipid syndrome. Other conditions that may cause arterial and venous thrombosis include paradoxical embolism, hyperhomocysteinemia, hyperviscosity syndrome, malignancy, concurrent vascular disease, heparin-induced thrombocytopenia/thrombosis (HIT), myeloproliferative disorder, PNH, and disseminated intravascular coagulation (DIC) (Table 12.2). Recurrent thrombosis despite therapeutic anticoagulation raises suspicion for antiphospholipid syndrome, cancer, DIC, Trousseau's syndrome, HIT, and structural defects.

Testing for thrombophilia in patients taking anticoagulation or who have acute thrombosis or other inflammatory processes may provide misleading results (Table 12.3). In general, any testing should be performed selectively and ideally delayed until after the acute phase (first 1–3 months) following a VTE event.

Antiphospholipid Antibody Syndrome

The antiphospholipid antibody syndrome (APS) is due to the acquisition of antibodies against phospholipids or phospholipid-binding proteins. Clinically APS may include any combination of arterial thrombosis, venous thrombosis, and recurrent pregnancy loss; it may also cause small-vessel disease and preterm delivery associated with preeclampsia or fetal growth restriction. Unlike other causes of VTE, APS may cause complications in any vascular bed and can (rarely) manifest catastrophically with multiorgan failure (Ruiz-Irastorza et al. 2010). Diagnosis is based on occurrence of clinical manifestations along with

Table 12.2 Causes of arterial and venous thromboembolism

Causes of arterial and venous thromboembolism
Hyperviscosity syndromes Sickle cell Multiple myeloma Waldenstrom's macroglobulinemia
Antiphospholipid syndrome
Hyperhomocysteinemia
Heparin-induced thrombocytopenia and thrombosis (HITT)
Disseminated intravascular coagulation (DIC)
Cancer
Paradoxical embolism
Popliteal artery aneurysm
Behcet's disease
Thromboangiitis obliterans (Buerger's disease)
Paroxysmal nocturnal hemoglobinuria
Nephrotic syndrome
Inflammatory bowel disease
Myeloproliferative disorders
Polycythemia vera
Essential thrombocytosis

Table 12.3 Caveats in laboratory testing for thrombophilia

	Confounding factors		
Assay for laboratory thrombophilia	Acute thrombosis	Heparin therapy	Coumadin therapy
Antithrombin	Can be lowered	Lowered	May be increased
Lupus anticoagulant	Potential false positives and false negatives	False positives/false negatives reported	False positives possible
Protein C	Can be lowered	No effect	Lowered
Protein S	Can be lowered	No effect	Lowered
Factor V Leiden mutation (PCR)	No effect	No effect	No effect
Prothrombin mutation (PCR)	No effect	No effect	No effect

persistently positive antibodies (beta 2 glycoprotein and anticardiolipin antibodies) or lupus anticoagulant on laboratory testing performed at least 12 weeks apart (Miyakis et al. 2006).

Probability of APS varies depending on the population. APS is more common in young to middle-aged women and in patients with autoimmune disorders, particularly systemic lupus erythematosus (SLE).

While antiphospholipid antibodies may be detected in about a quarter of patients with VTE who have positive laboratory testing for thrombophilia (Roldan et al. 2009), it is *persistent* positivity of antibodies or lupus anticoagulant assays that increases risk of future VTE. Otherwise healthy individuals with persistently positive antiphospholipid antibodies have odds for VTE of up to about ten times that in the general population; this risk varies with antibody titres and antibody category (Wahl et al. 1998). Risk is substantially higher in the setting of autoimmune disease in general and systemic lupus erythematosus (SLE) in particular; nearly 40 % of SLE patients with antiphospholipid antibodies develop VTE (Ruiz-Irastorza et al. 2010; Love and Santoro 1990).

Among antiphospholipid antibody groups, lupus anticoagulant remains the strongest predictor of thrombosis. Risk is further elevated in the presence of multiple categories of elevated antiphospholipid antibodies (Ruiz-Irastorza et al. 2011). Testing guidelines have evolved; as of this writing, recommended evaluation for antiphospholipid antibodies includes lupus anticoagulant, anticardiolipin IgG and IgM antibodies, and beta-2-glycoprotein IgG and IgM antibodies. Testing should be repeated at least 12 weeks from the initial assay to demonstrate persistence (Ruiz-Irastorza et al. 2010). Accuracy of testing is confounded by concurrent anticoagulation, particularly for lupus anticoagulants, with both false positive and false negatives being reported.

Protein S Deficiency

Protein S, a vitamin K-dependent clotting protein, complexes with activated protein C to inactivate Factors Va and VIIIa. It exists both as a free protein (60 %) and as a complex with C4b-binding protein (C4b-BP) and may be quantitatively measured by free and total antigen levels. Deficiencies of protein S activity may be due to qualitatively low levels of protein S itself (type I), dysfunctional protein S leading to a qualitative deficit with normal antigen levels but decreased activity (type II), or quantitative deficit caused by abnormal or excessive C4b-BP binding, leading to low free-protein S antigen levels but normal total levels (type III). Most of the over 100 known mutations (93 %) cause quantitative (type I or III) deficiencies (Moll 2006).

Prevalence of protein S deficiency was 7.5 % in a study of 4,494 patients with VTE who underwent thrombophilia testing and was slightly higher (9 %) in the group of patients under age 50 (Roldan et al. 2009). However, a number of concurrent conditions may decrease protein S concentrations, leading to misdiagnosis of inherited protein S deficiency. These include concurrent use of a vitamin K antagonist, liver disease, oral contraceptive use, pregnancy, nephrotic syndrome, acute thrombosis, and DIC. Activated protein C resistance as seen in patients with Factor V Leiden mutation can lead to falsely low protein S functional assay value. Sickle cell trait can cause decreased protein S activity.

Protein C Deficiency

Protein C, a vitamin K-dependent protein, complexes with protein S when activated and inactivates Factors Va and VIIIa. Protein C deficiency may involve a quantitative deficit in protein C (type I, about 85 % of cases) or a qualitative deficiency with low activity (type II) (Moll 2006). The prevalence of protein C mutations in the population has been documented at 1 in 500 to 1 in 600 (Tait et al. 1995), but protein C deficiency is diagnosed in only around 4 % of patients with VTE (Roldan et al. 2009). While numerous mutations may cause deficiency of protein C, homozygous mutation causes catastrophic complications at birth and thus is unlikely to be diagnosed later in life. Testing for protein C antigen levels identifies patients with quantitative (type I) deficiency, but testing for protein C activity is needed to identify both qualitative and qualitative deficits.

Unfortunately a number of concurrent factors may lead to erroneous diagnosis of decreased protein C activity. The most common of these is concomitant use of a vitamin K antagonist, which decreases protein C activity since the protein is vitamin K dependent; testing must be done after 2–3 weeks off anticoagulation. High levels of Factor VIII and presence of lupus anticoagulant may produce falsely decreased protein C activity levels (Moll 2006).

Antithrombin Deficiency

Antithrombin (AT, previously referred to as antithrombin III) is a natural anticoagulant which prevents clotting by inhibiting thrombin and other clotting proteins. Heparin accelerates antithrombin's normally low level of inhibitory activity; hence, AT's role is as a heparin cofactor. Clinical antithrombin deficiency may be due to low levels of antithrombin (type I) or a dysfunctional protein leading to normal levels but low activity (type II). Deficiency of antithrombin is inherited in an autosomal dominant pattern with variable penetrance, and over 100 mutations affecting antithrombin production or activity have been identified (Moll 2006).

Although uncommon (found in 1:600 in the general population (Tait et al. 1994) and 1–3 % of patients with VTE) (Roldan et al. 2009; Pabinger et al. 1992; Heijboer et al. 1990), deficiency of antithrombin carries strong risk for VTE. As with protein C and protein S, defects in antithrombin activity may be missed if only antigen level is tested; therefore, activity level is also recommended. Antithrombin levels may be decreased in the settings of acute thrombosis and heparin administration. Levels may also be decreased by impaired synthesis in liver failure and by increased excretion in nephrotic syndrome. Warfarin administration may lead to increased AT levels, causing a falsely normal result (Moll 2006).

Hyperhomocysteinemia

Elevated levels of plasma homocysteine are associated with both venous and arterial thrombosis. However, the causal relationship between homocysteine and VTE remains unclear. While mutations in the methylenetetrahydrofolate reductase (MTHFR) gene can cause hyperhomocysteinemia, they have not been shown in meta-analyses to be associated with VTE events in the absence of hyperhomocysteinemia (Den Heijer et al. 2005; Ray et al. 2002). While testing for hyperhomocysteinemia may help clarify a patient's risk for VTE and other vascular events, testing for MTHFR mutations is not currently recommended. The utility of testing for hyperhomocysteinemia is also limited by the fact that lowering levels does not decrease risk for future events.

Factor V Leiden and Prothrombin Gene Mutations

The Factor V Leiden mutation, which confers resistance to activated protein C and involves a single amino acid exchange in position 506 of the Factor V molecule, is the most common identified inherited thrombophilia. It is found in about 5 % of the US American population, with lower prevalence among groups without European Caucasian ancestry (Moll 2006). While relative risk of VTE in people heterozygous for Factor V Leiden is about three times the risk in people without the mutation, absolute risk remains fairly low (<1 % per year). Risk is about 18-fold higher in people homozygous for the mutation, however (Segal et al. 2009).

The prothrombin 20210 point mutation is a gain-of-function mutation leading to increased levels of circulating prothrombin, which in turn stimulates increased generation of fibrin. Heterozygosity for the mutation has not convincingly or consistently been shown to increase risk of VTE in absence of other risk factors, but adds significantly to risk when other factors are present (Segal et al. 2009). Homozygosity for the prothrombin mutation is rare, and thus, data are limited regarding associated risk; limited case studies suggest substantial phenotypic variation (Bosler et al. 2006), emphasizing the contributing roles of other risk factors for VTE.

Heterozygosity for Both Factor V Leiden and Prothrombin Gene Mutations

Although heterozygosity for Factor V Leiden or the prothrombin gene 20210 mutation

confers only a modestly increased risk for VTE, combined heterozygosity confers substantially greater risk. A pooled analysis of case–control studies found odds ratios for VTE of 4.9 and 3.8 for Factor V Leiden and prothrombin G20210A mutations, respectively, but an odds ratio of 20 in double heterozygotes (Emmerich et al. 2001).

Other Heritable and Acquired Thrombophilias

A number of other conditions including but not limited to excess Factor VIII levels, dysfibrinogenemia, heparin cofactor II deficiency, and plasminogen deficiency have been determined to be independent risk factors for VTE (Jenkins et al. 2012), but the importance of these in clinical practice remains to be determined.

Absence of a known major laboratory thrombophilia does not rule out the strong possibility that a patient who has had an unprovoked event carries an ongoing tendency to have recurrent VTE, as our hypercoagulability panel is limited and in constant evolution.

Medications

Clinical Vignette 3

An otherwise healthy 22-year-old woman who started taking a combined oral contraceptive pill 3 months ago presents to urgent care with acute dyspnea and is found to have a pulmonary embolism.

Numerous medications may contribute to VTE risk; here we highlight the major categories of hormonal compounds, cancer therapeutics, medications affecting red blood cell mass, and antipsychotic medications.

Hormonal Compounds

Hormonal compounds contributing to VTE risk include estrogen-containing oral contraceptives and hormone replacement therapy, as well as estrogen-modulating cancer therapeutics including tamoxifen and raloxifene.

Combined Oral Contraceptives (COCs)

The overall odds ratio for VTE in women taking estrogen-containing oral contraceptive pills versus those not taking COCs is around 3–4 and has not changed significantly in the years since OCPs began to be used. Risk increases with dose of estrogen and is greatest in women taking COCs containing the third-generation progestin desogestrel (Manzoli et al. 2012; Gomes and Deitcher 2004). In the Leiden Thrombophilia Study, COC users had 3.8-fold greater odds of VTE than non-COC users. This odds ratio increased to 8.7 in women using COCs containing desogestrel, compared to 2.2–3.8 for women using COCs with first- or second-generation progestins (van der Meer et al. 1997). Risk appears to be highest during the first several months of use and is higher for older than for younger women, reflecting the underlying increase in baseline risk with increasing age (Gomes and Deitcher 2004).

Risk is substantially higher for women taking COCs who also have Factor V Leiden or prothrombin G20210A mutations. In the Leiden Thrombophilia Study, women with concurrent Factor V Leiden mutation and COC use had 28.5 times greater odds of VTE than women with neither risk factor (Roldan et al. 2009); subsequent studies have suggested up to 99-fold increased risk (Gomes and Deitcher 2004). A case–control study of 477 women found an odds ratio of 16.3 for VTE in women taking COCs with the prothrombin G20210A mutation compared to women without the mutation who were not taking COCs (Martinelli et al. 1999). These risks have been somewhat lower in subsequent studies including a pooled analysis of case–control studies which found an OR of 10.25 for VTE in women with Factor V Leiden mutations taking OCPs and 7.14 in women with the prothrombin gene mutation taking OCPs (Emmerich et al. 2001).

The transdermal contraceptive system is associated with higher levels of circulating estrogen than oral contraceptives. Available data suggest that the VTE risk associated with the transdermal

contraceptive patch is at least equal to, and perhaps up to 2.4 times greater than, the risk from combined oral contraceptives (Jick et al. 2007; Cole et al. 2007). Risk associated with the vaginal contraceptive ring has not yet been established.

Progestin-Only Contraception

Limited data are available regarding risk associated with progestin-only hormonal contraception, including oral and injectable methods. While several studies have demonstrated slightly increased risk, this risk has not reached statistical significance in individual studies or in a 2009 meta-analysis demonstrating a relative risk for VTE of 1.45 (95 % CI 0.92, 2.26) for women using progestin-only contraceptives (Gomes and Deitcher 2004; Blanco-Molina et al. 2012; Bergendal et al. 2009; Barsoum et al. 2010). Similarly, use of the progestin megestrol acetate as an appetite enhancer has been associated with a slight but nonsignificant increase in VTE risk (Barsoum et al. 2010). Extended follow-up from a large study of hormonal therapy for postmenopausal women did, however, show a small but significant increase in VTE risk among women taking estrogen and progestin compared to those taking estrogen alone (Curb et al. 2006).

Hormone Replacement Therapy (HRT)

Two large studies, the Women's Health Initiative and the Heart and Estrogen–Progestin Replacement Study, noted increased risk for VTE among women taking hormonal replacement therapy with estrogen alone or combined estrogen and progestin (Heiss et al. 2008; Hulley et al. 2002). Multiple smaller studies and a meta-analysis have provided supporting data, with odds ratios around 2–3 for VTE in women taking HRT (either unopposed estrogen or combined estrogen–progestin) (Beral et al. 2002; Canonico et al. 2008); increased dose of estrogen correlates with higher VTE risk (Renoux et al. 2010). Risk is highest during the first year of treatment and returns to baseline following cessation of therapy. Despite consistent evidence confirming the association between oral estrogen and VTE, transdermal estrogen for hormone replacement does not appear to increase risk for VTE (Olie et al. 2011a, b).

Estrogen-Modulating Anticancer Agents

The selective estrogen receptor modulators (SERMs) tamoxifen and raloxifene are associated with a two- to threefold increase in VTE risk. Additional cancer therapeutics with estrogen-modulating activities, including the aromatase inhibitor anastrozole, have also been shown to increase VTE risk, albeit to a lesser extent (Deitcher and Gomes 2004).

Other Cancer Therapeutics

Cancer chemotherapy has been consistently associated with VTE risk above that attributable to malignancy alone. Compared with an annual VTE incidence of around 1 per 200 in all patients with cancer (Lee and Levine 2003), annual VTE incidence in patients undergoing chemotherapy has been reported as up to 10.9 % (Otten et al. 2004; Khorana et al. 2005). Certain chemotherapeutic agents including lenalidomide and thalidomide for myeloma have been particularly associated with VTE risk; phase 3 trials of these medications showed a 2- to 14-fold higher incidence of VTE with thalidomide and a two- to ninefold greater incidence with lenalidomide, compared with control arms (Bennett et al. 2006).

Antipsychotic Medications

Several studies have demonstrated a small but significantly increased risk for VTE associated with antipsychotic medications, with odds ratios around 2. Risk may be greatest with use of low-potency first-generation antipsychotics and with clozapine, although the confounding effects of immobility, obesity, and other factors associated with VTE and with administration of antipsychotic medications have limited clear establishment of this risk (Hagg et al. 2009; Jonsson et al. 2009).

Transfusions and Erythropoiesis-Stimulating Agents

Red blood cell transfusions, platelet transfusions, and erythropoiesis-stimulating agents are associated with a slightly but significantly increased risk for VTE, although their independent contributions to risk are difficult to assess as additional factors are often present including concurrent

acute illness, malignancy, and chemotherapeutic agents (Rizzo et al. 2010; Khorana et al. 2008; Tonelli et al. 2009).

Discussion of Clinical Vignette 3

Although the overall risk for VTE in an otherwise healthy 22-year-old woman is low (around 1 in 10,000), risk is moderately elevated by a factor of 3–4 by use of combined oral contraceptive pills. Given her age and absence of other provoking factors, it is likely that this young woman also has a Factor V Leiden or prothrombin gene mutation. Although genetic testing is unlikely to affect recommended duration of anticoagulation (neither mutation would commit her to indefinite anticoagulation as long as she discontinues her hormonal contraceptive), testing may be warranted if she is considering pregnancy in the future. Evaluation for APS should be considered given implications for anticoagulation monitoring, recurrence risk, and management during pregnancy.

Malignancy

Risk of VTE is elevated about four- to sevenfold for patients with cancer (Lee and Levine 2003; Piccioli and Prandoni 2011; Rickles and Levine 2001) compared to the general population. About 10–15 % of patients with overt cancer will have VTE at some point during the course of their disease, although risk varies based on extent of disease, tumor type, and presence of numerous other factors that elevate VTE risk in cancer patients including hospitalization, surgery, immobilization, chemotherapy, and central venous access (Rickles and Levine 2001). Mucin-producing adenocarcinomas including pancreas, lung, stomach, and adenocarcinoma of unknown primary appear the most likely to cause thrombosis. However, the most common tumors found in patients with DVT, reflective of overall prevalence in the general population, are prostate, colon, lung, and brain in men and breast, lung, and ovary in women (Lee and Levine 2003). Incidence of thrombosis in hematologic malignancies was previously thought lower than for solid tumors, but is now thought similar. As with solid tumors, risk in hematologic malignancies is further increased by chemotherapeutic regimens (thalidomide and lenalidomide for myeloma in particular), central venous catheters, frequent hospitalizations, and comorbidities (Elice and Rodeghiero 2012).

VTE is a common complication among patients with known cancer, but may also present as a first manifestation of occult malignancy. Up to 10 % of patients with idiopathic VTE may be diagnosed with malignancy within 5–10 years of VTE presentation; for most of these patients, diagnosis of cancer is established within the first 6 months after presentation (Lee and Levine 2003; Piccioli et al. 2006). Most patients who present with VTE and have occult malignancy have some clinical abnormality suggestive of malignancy at the time of VTE diagnosis. Extensive screening for all patients presenting with idiopathic DVT is generally not warranted (Hettiarachchi et al. 1998; Piccioli et al. 2006). Identification of occult malignancy has implications for treatment, as cancer-related VTE is generally treated with low-molecular-weight heparin, while non-cancer patients are transitioned to warfarin.

Thromboembolic disease in malignancy is further addressed elsewhere in this book.

Other Hematologic Disorders

Risk of VTE is increased in patients with benign monoclonal gammopathy of undetermined significance (MGUS) with a risk elevated around 2–3 times that in control subjects. Occurrence of VTE has not been associated with progression of MGUS to myeloma or with monoclonal protein levels (Elice and Rodeghiero 2012). Myeloproliferative disorders, particularly essential thrombocytosis (ET) and polycythemia vera (PV), confer increased risk for both arterial and venous thrombosis; presence of venous thromboembolism at unusual sites (cerebral sinuses or splanchnic veins) or at a young age may provide clues to an underlying myeloproliferative disorder.

In patients with suggestive laboratory or clinical findings, testing for the JAK2 mutation may be helpful (Landolfi et al. 2008). Paroxysmal nocturnal hemoglobinuria, which also predisposes to both arterial and venous events, also more commonly presents in the abdominal veins than in the lower extremities (Ray et al. 2000).

Pregnancy

Risk of VTE increases by about fourfold during pregnancy but is most pronounced in the postpartum period when it increases 14- to 84-fold. Hypercoagulability during and following pregnancy is likely due to alterations in hemostatic mechanisms to prevent bleeding; as pregnancy progresses, stasis and venous compression from the gravid uterus also contribute to risk (Lussana et al. 2012). Pelvic vein thrombosis, an otherwise rare manifestation of VTE, accounts for about 10 % of DVT during pregnancy, and the postpartum period DVT during pregnancy more commonly occurs in the left leg than the right, likely due to compression of the left iliac vein by the right iliac artery, i.e., the May–Thurner syndrome (Chan et al. 2010; James et al. 2005).

Risk is dramatically increased in the presence of concurrent thrombophilia, particularly antithrombin deficiency, Factor V Leiden mutation (homozygous or heterozygous), or prothrombin G20210A mutation (homozygous or heterozygous). Additional significant risk factors contributing to peripartum VTE include hemorrhage, transfusion, prior VTE, preeclampsia, and postpartum infection (Lussana et al. 2012; James et al. 2006; Bates et al. 2012). Thrombosis risk associated with pregnancy and the postpartum period is addressed elsewhere in this book.

Minor Risk Factors

Immobility and Travel

Immobilization is a minor but significant risk factor for VTE. Although a number of studies have assessed the effect of immobilization on VTE risk, varying definitions of immobilization in terms of time period and degree of immobility make exact quantification of risk difficult. Nonetheless there is general consensus that risk of VTE increases during periods of significant inactivity (such as total bed rest or bed rest with bathroom privileges) lasting over 3 days, and that risk is approximately doubled (Anderson and Spencer 2003; Pottier et al. 2009; Hull 2012). Incidence of VTE in chronically immobilized outpatients is increased above that for ambulatory patients, but there are few studies available to further quantify risk. While annual incidence of VTE in nursing home patients is around 1 %, post-acute care patients have an annual incidence of VTE between 1.0 and 2.4 %, approaching that of hospitalized patients (Kahn et al. 2012).

Although much research on immobilization and VTE has been focused on inpatients or chronically immobilized outpatients, prolonged immobilization among otherwise healthy outpatients is also a recognized, albeit minor, risk factor. Relatively minor isolated limb injury with ensuing immobilization, with or without plaster casts or other orthopedic devices, may trigger VTE (Nilsson-Helander et al. 2009; Cogo et al. 1994). Prolonged sitting at work may also contribute to VTE (Beasley et al. 2003; West et al. 2008; Healy et al. 2010); however, this is unlikely to happen in the absence of other predisposing factors.

Travel

Prolonged travel increases risk for VTE. Case–control studies have demonstrated odds ratios between 1.5 and 4 for VTE following travel by any modality lasting greater than 3–4 hours (Gallus and Goghlan 2002). Particular attention has been given to VTE risk associated with air travel given concern about the additional effect of the hypobaric environment during flight. Estimates of incidence of travel-related VTE vary, but overall risk per travel episode is likely around 0.05 % (Philbrick et al. 2007; Kuipers et al. 2007). Travelers on flights under 4–6 hours in duration do not have significantly elevated risk, and risk increases incrementally with duration of travel over 6 hours (Philbrick et al. 2007). While overall risk is low, travelers with additional risk factors have substantially increased susceptibility

to travel-related VTE (Cannegieter 2012; Kuipers et al. 2009). Frequent ambulation and calf muscle exercise are recommended for all long-distance travelers; for those at elevated risk of VTE due to recent surgery, trauma, malignancy, prior VTE, pregnancy, estrogen use, advanced age, or known thrombophilia, graduated compression stockings have been demonstrated to decrease VTE risk and are recommended (Kahn et al. 2012).

Additional Medical Conditions

A wide range of autoimmune diseases have been shown to confer elevated risk for thromboembolism. Inflammatory bowel disease carried a standardized incidence ratio of 10–12 for pulmonary embolism in one longitudinal Swedish study, and the overall incidence ratio for PE for all autoimmune diseases studied was 6.4 (Zoller et al. 2012). The nephrotic syndrome carries well-documented risk for both venous (including renal vein) and arterial thromboembolic disease; mechanisms likely include preferential loss of antithrombin and other proteins involved in inhibition of systemic hemostasis (Singhal and Brimble 2006; Mahmoodi et al. 2008).

There is increasing awareness of elevated VTE risk in a number of chronic renal, cardiac, and pulmonary diseases. Patients with chronic kidney disease appear to have slightly increased risk for VTE (Wattanakit and Cushman 2009; Mahmoodi et al. 2012). Congestive heart failure is associated with increased VTE risk in the inpatient and outpatient settings; this independent association is particularly strong in younger (under age 40) patients with CHF (Cogo et al. 1994; Beemath et al. 2006). Patients with chronic obstructive pulmonary disease have increased VTE risk as well and may face increased morbidity and mortality from pulmonary emboli due to diminished cardiopulmonary reserves (Shetty et al. 2008).

While many acute infections transiently increase VTE risk, chronic infections also may lead to sustained risk elevation. Case–control data have suggested two- to tenfold greater incidence of VTE in people living with AIDS compared to matched controls; this risk may be exacerbated by concurrent infections and presence of additional risk factors (Auerbach and Aboulafia 2012).

Overlap with Traditional Vascular Risk Factors

There is increasing recognition of the overlap between risk factors for venous and arterial vascular disease.

Obesity has long been recognized as a risk factor for VTE; it may double risk of a first VTE, and excess body weight increases risk for VTE recurrence (Allman-Farinelli 2011; Eichinger et al. 2008). Additional risk factors for atherosclerotic vascular disease have now also been shown to modestly but significantly increase VTE incidence (odds ratios between 1 and 2); these include tobacco use, hyperlipidemia, hypertension, and diabetes mellitus (Ageno et al. 2008).

VTE in Hospitalized Patients

Clinical Vignette 4

A 72-year-old woman with morbid obesity and a history of congestive heart failure is admitted to the hospital with pneumonia. She is ambulatory and has been able to use the bathroom and take short walks with the physical therapist.

Hospitalization and long-term nursing home care are risk factors for VTE. Incidence of VTE in hospitalized patients has been reported as up to 10–30 % (Cohen et al. 2005). Critical illness carries VTE risk above that found for medical hospitalized patients (Cook et al. 2005). For medical inpatients, independent risk factors for VTE include active cancer, increased age, increased body mass index, paresis due to neurologic disease, fracture, chronic kidney disease, prior deep or superficial venous thrombosis, and prolonged immobility (Barbar et al. 2010; Heit 2008).

Table 12.4 Padua risk score for venous thromboembolism in hospitalized patients

Padua prediction model (high risk for VTE: ≥4 points)	
Risk factor	Points
Active cancer[a]	3
Previous VTE (not superficial)	3
Reduced mobility: bed rest/bathroom privileges for at least 3 days	3
Known thrombophilia[b]	3
Trauma or surgery in past month	2
Age at least 70	1
Congestive heart failure or respiratory failure	1
Acute infection and/or rheumatologic disorder	1
Body mass index at least 30	1
Ongoing hormonal treatment	1

In an initial validation study, VTE developed in 11 % of high-risk patients (score ≥4) who did not receive pharmacologic prophylaxis and in 2.2 % of high-risk patients who received pharmacologic prophylaxis

Adapted with permission from Barbar S, Noventa F, Rossetto V, Ferrari A, Brandolin B, Perlati M et al. A risk assessment model for the identification of hospitalized medical patients at risk for venous thromboembolism: the Padua Prediction Score. Journal of Thrombosis and Haemostasis: JTH. 2010;8(11):2450–7

[a]Local or distant metastases or chemotherapy or radiotherapy in past 6 months

[b]Antithrombin, protein C, protein S, Factor V Leiden, prothrombin 20210 mutation20210 mutation, antiphospholipid syndrome

For surgical inpatients, risk factors include active malignancy and type of surgery; additional independent risk factors include intensive care admission for at least 6 days, presence of a central venous catheter, increased BMI, varicose veins, and infection (Heit 2008).

Risk scores may help stratify hospitalized patients and determine when VTE prophylaxis is warranted. For noncritically ill hospitalized medical patients, the Padua score can be used to estimate VTE risk (Barbar et al. 2010) (Table 12.4); for surgical patients, the Caprini score can be used (Caprini 2010). Other risk scores that are not as widely validated include the Vienna score for risk stratification in outpatients with cancer (Thaler et al. 2012; Khorana 2011) and the QThrombosis score, a recently developed model to predict VTE risk in primary care patients in the UK (Hippisley-Cox and Coupland 2011).

Note that the relative weights of certain risk factors may differ based on the populations addressed by specific risk scores and classification schemes. For instance, among noncritically ill hospitalized medical patients, active malignancy and known thrombophilia are major risk factors for VTE based on the Padua model (Table 12.4), although in Table 12.1 these are listed as "moderate" risk factors based on studies reflecting risk in the overall population. Likewise, *major* surgery and trauma are major risk factors for VTE in the overall population as reflected in Table 12.1, but "recent trauma or surgery" is a moderate risk factor in the Padua model, likely reflecting inclusion of nonmajor surgery or trauma and its impact on specifically medical, nonsurgical inpatients. Although risk scores can be quite helpful, it is important to keep in mind that they represent a simplification of the risk associated with heterogeneous categories such as surgery, thrombophilias, and malignancy, and each patient's individual history must be taken into account for full assessment of VTE risk.

Discussion of Clinical Vignette 4

For medical inpatients, the Padua risk stratification score (Table 12.4) may be a useful tool to identify patients who will benefit most from pharmacologic VTE prophylaxis. In a study of 1,180 patients, VTE developed in 0.3 % of those with scores less than 4, in 2.2 % of those with scores 4 or greater who received pharmacologic prophylaxis, and in 11 % of patients with scores 4 or greater who did not receive prophylaxis (Barbar et al. 2010). Although this patient is ambulatory, her Padua risk score is 4 (1 point each for age, obesity, congestive heart failure, and acute infection), placing her in the "high-risk" category of hospitalized patients for whom administration of prophylactic low-molecular-weight heparin or unfractionated heparin may provide a meaningful reduction in VTE risk.

Anatomic Anomalies

Aberrant anatomic structures causing VTE are infrequent in the population, yet recognition is important as it can have implications for the effectiveness of anticoagulation and lead to correction and mitigation of future risk. In May–Thurner syndrome, compression of the left common iliac vein by the right common iliac artery leads to development of chronic venous insufficiency and iliofemoral DVT (Kim and Choi 2006). Paget–Schroetter syndrome, an unusual cause of upper extremity DVT, involves axillary subclavian thrombosis that occurs in the setting of excessive arm activity in the presence of compressive elements in the thoracic outlet (Urschel and Razzuk 2000). Congenital anomalies of the inferior vena cava (IVC) include narrowing, duplication, or even absence of the IVC and should be suspected in young patients with bilateral, unprovoked DVT (Chee et al. 2001). Popliteal venous aneurysms may have internal thrombosis which can cause pulmonary emboli (Bergqvist et al. 2006); popliteal arterial aneurysms may also cause VTE via compression of the popliteal vein.

Conclusion

Determining the etiology of a thrombotic event is important to both provider and patient. Identifying removable or modifiable risk factors and permanent risk factors that will influence duration or type of therapy is most critical. A stepwise approach to exploring etiology may be most helpful and economical, focusing first on classifying an event as provoked or unprovoked and next on what further clinical or laboratory evaluation may be warranted based on patients' age, medical history, and personal preferences. Providers must have a knowledge of these risk factors and be committed to educating patients on risk factor modification and about the warnings signs and symptoms of DVT and PE to help reduce incidence and mortality associated with VTE.

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Clotting Around Catheters and Devices

13

Natalie S. Evans, Manoj K. Dhariwal, and Lee Joseph

Clinical Vignette 1

A 54-year-old man presents to the emergency department with a 3-day history of right upper extremity tenderness and swelling. Seven days ago, a peripherally inserted central catheter (PICC) was placed for 3 weeks of intravenous antibiotic therapy to treat methicillin-resistant *Staphylococcus aureus* osteomyelitis that developed after open reduction and internal fixation of a left femur fracture. He denies shortness of breath, chest pain, and fever. Physical examination is remarkable for right upper extremity edema, tenderness, warmth, and erythema around the PICC site.

The first-year emergency department resident suspected upper extremity deep vein thrombosis and performed a bedside ultrasound, which showed absence of color flow and compressibility in the right axillary and subclavian veins adjacent to the PICC. What is the best next step?

1. Remove the PICC.
2. Admit the patient, start anticoagulation, continue antibiotics, and continue to use the PICC.
3. Send the patient home on current antibiotics, and recommend arm elevation with warm compresses and anti-inflammatory/analgesic gel topically.
4. Admit the patient, start anticoagulation, continue antibiotics, and remove the PICC.

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Catheter-Related Thrombosis

Introduction

Venous thromboembolism (VTE) related to central venous catheters (CVCs) and devices is a common complication encountered in clinical practice that can lead to significant morbidity and mortality (Joffe et al. 2004; van Rooden, Molhoek 2004). Catheter-related thrombosis (CRT) is associated with increased risk of pulmonary embolism (PE), catheter-related infection, post-thrombotic

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syndrome, and difficulty obtaining later vascular access (van Rooden, Molhoek et al. 2004; Baskin et al. 2009; Elman and Kahn 2006; Owens et al. 2010).

Epidemiology

The incidence of CRT varies depending on the type of catheter and its site of insertion, technical issues at insertion, diagnostic test used, and patient population (Acedo Sanchez et al. 2007). Up to a third of patients with indwelling catheters develop symptomatic CRT (Agnelli and Verso 2006; Camara 2001; van Rooden, Rosendaal et al. 2004; Verso and Agnelli 2003). Approximately 15 % of patients in medical intensive care units diagnosed with any DVT have catheter-related UEDVT (Hirsch et al. 1995). Of patients with cancer and CVC followed prospectively for up to a year, 4.3 % developed symptomatic CRT, with thrombosis diagnosed on average within 30 days after catheter insertion (Lee et al. 2006).

Inherited defects of coagulation factors may play a role in the development of CRT. In one study of more than 250 hospitalized patients with CVCs who were followed prospectively with Doppler ultrasound examinations, 30 % developed CRT. The presence of the factor V Leiden or prothrombin gene mutations was a risk factor for CRT, with a relative risk of 2.7 (95 % confidence interval 1.9–3.8) (van Rooden, Rosendaal et al.2004).

Pathophysiology

The pathophysiology of CRT is best explained by Virchow's triad, which describes the three key components of thrombus formation: endothelial injury, circulatory stasis, and hypercoagulable states (Fig. 13.1). First, intimal damage from CVC insertion or malpositioning exposes tissue factor, leading to platelet aggregation and thrombus formation by means of activation of the coagulation cascade. Irritation from drugs infused via the catheter may also lead to intimal damage. Second, venous stasis occurs as a result of immobilization, reduced cardiac output, and turbulent flow secondary to displacement of the faster

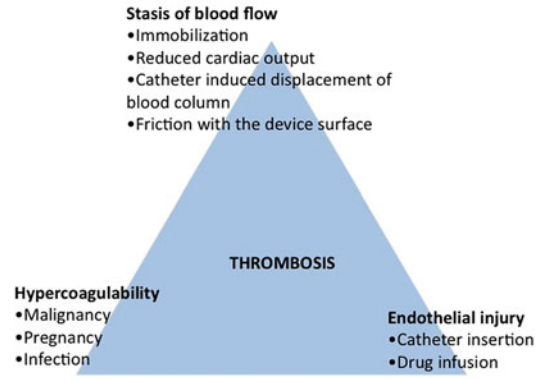

Fig. 13.1 Virchow's triad

moving central blood column by an indwelling venous catheter. Third, hypercoagulability is an important contributor to thrombus formation. Commonly encountered hypercoagulable states in CRT include malignancy, pregnancy, and infections (Verso and Agnelli 2003; Holmgren et al. 2008; Schmidt et al. 2012).

Though fibrin sheath formation around the catheter, with an incidence as high as 87 %, and thrombus formation within the catheter lumen are common, these events do not predict subsequent development of venous thrombosis (De Cicco et al. 1997; Kuter 2004).

Risk Factors

Patient-Related Risk Factors

Hypercoagulable states such as malignancy, in particular lung adenocarcinomas and ovarian tumors, and especially cancer chemotherapy, including such drugs as thalidomide, lenalidomide, tamoxifen, fluorouracil, anthracycline, cisplatin, hematopoietic growth factors, and antiangiogenic agents, are strongly associated with CRT (Agnelli and Verso 2006; Lee et al. 2006; Kuter 2004; Andtbacka et al. 2006; King et al. 2006; Prandoni and Bernardi 1999). Other patient-related risk factors include extremes of age; body mass index greater than 28; female sex; chronic illnesses such as lupus, inflammatory bowel disease, stroke, and end-stage renal disease; hip and knee replacement surgery; trauma and spinal injuries; and prior history of venous thromboembolic disease (Joffe et al. 2004; van Rooden, Rosendaal

et al.2004; King et al. 2006; Davidson 1999; Ong et al. 2006; Otten et al. 2003).

As mentioned previously, the factor V Leiden and prothrombin gene mutations are known to predispose to UEDVT, but data are sparse for their contribution to CRT. Nevertheless, a meta-analysis involving 1,000 patients and examining the association between factor V Leiden and prothrombin gene mutations and CRT showed a pooled odds ratio of 4.6 (95 % confidence interval 2.6–8.1) (Dentali et al. 2008).

Catheter-Related Risk Factors

Suboptimal placement of the catheter tip, difficult or traumatic catheter insertion, prior catheter placements, PICCs, multi-lumen devices, larger diameter of catheter, and presence of additional vascular devices such as pacemakers may increase the risk for CRT (Lee et al. 2006; Kuter 2004; Ascher et al. 2005; Cortelezzi et al. 2005; Cortelezzia et al. 2003; Grove and Pevec 2000; Male et al. 2003; Penney-Timmons and Sevedge 2004). PICCs may be particularly problematic. In a study examining intensive care unit patients using Doppler ultrasound and comparing CVCs with PICCs, nearly 30 % of PICC patients developed thrombosis, while less than 10 % of CVC patients developed thrombosis (Bonizzoli et al. 2011). In a recent systematic review of more than 5,000 cancer patients, those individuals with PICCs were more likely to develop CRT than those with implanted ports (Saber et al. 2011). Although PICCs typically are smaller in diameter than CVCs, they are longer, and they may completely fill the vein at the insertion site, leading to stagnation of blood and potential for thrombus formation. Underscoring this point, in more than 2,000 PICC insertions at a tertiary hospital, double-lumen and triple-lumen PICCs were associated with higher thrombosis rates than single-lumen PICCs (Evans et al. 2010). In another study examining catheters by diameter, catheters smaller than 3 French had no thrombosis, while 6 French catheters thrombosed in 10 % of cases (Grove and Pevec 2000).

There is an up to eightfold increase in the risk for CRT, phlebitis, or catheter mechanical dysfunction if the catheter tip is malpositioned (Verso and Agnelli 2003; Bona 2003; Racadio et al. 2001). In a study of 145 patients with indwelling catheters, those with optimally positioned catheter tip, either in the superior vena cava or at the junction of the superior vena cava and right atrium, had a CRT rate of just 9 %, compared with 45 % in patients with malpositioned catheter tips (Luciani et al. 2001). Subclavian insertion has the lowest incidence of thrombosis compared with other access sites (McGee and Gould 2003). Internal jugular venous access is the most common site of CRT, associated with a fourfold increased risk of thrombosis compared with subclavian insertion (Biffi et al. 2009; Major et al. 2008). CRT complicates 21.5 % of cases of femoral venous access compared with 1.9 % with subclavian venous access (Merrer et al. 2001).

Diagnosis

Clinical Manifestations

CRT is often asymptomatic (van Rooden, Rosendaal et al. 2004), but common clinical manifestations may include pain and swelling in the affected arm and palpable venous cords. Patients who develop chronic venous occlusion may have superficial venous collaterals on the chest wall and anterior shoulder. Patients who develop pulmonary embolism may complain of chest pain and be short of breath and tachycardic, and those with obstruction of the superior vena cava may have swelling or plethora of the face, neck, trunk, and arms, along with shortness of breath that may be positional.

Up to 30 % of otherwise asymptomatic patients may present with catheter malfunction, with inability to aspirate blood from the catheter (Baskin et al. 2009).

Diagnostic Studies

Though chest X-ray and D-dimer are quick, relatively inexpensive tests, they are not specific for diagnosing CRT. Duplex ultrasound, combining grayscale compression and Doppler waveform analysis, is the diagnostic test of choice. Thrombus may appear occlusive or non-occlusive

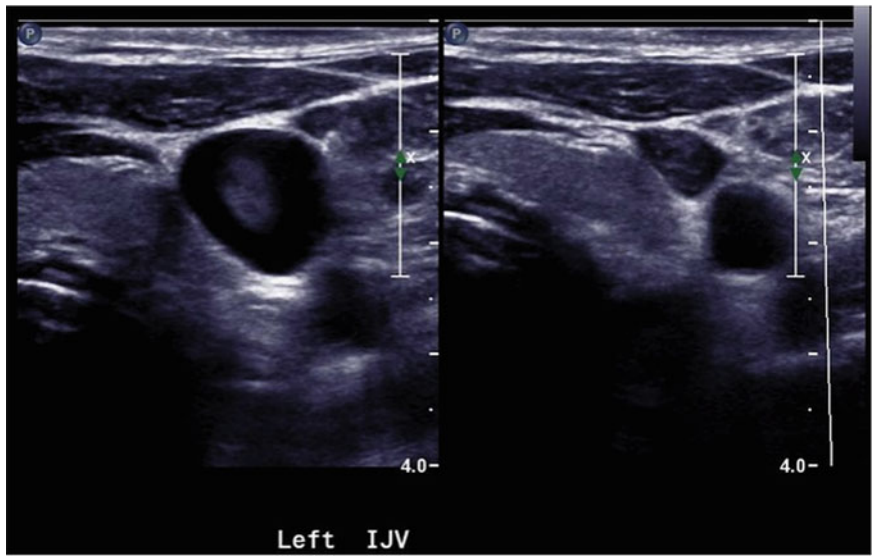

Fig. 13.2 In the *left panel*, ultrasonography shows the internal jugular vein filled with echogenic thrombus. In the *right panel*, the vein walls do not coapt with compression

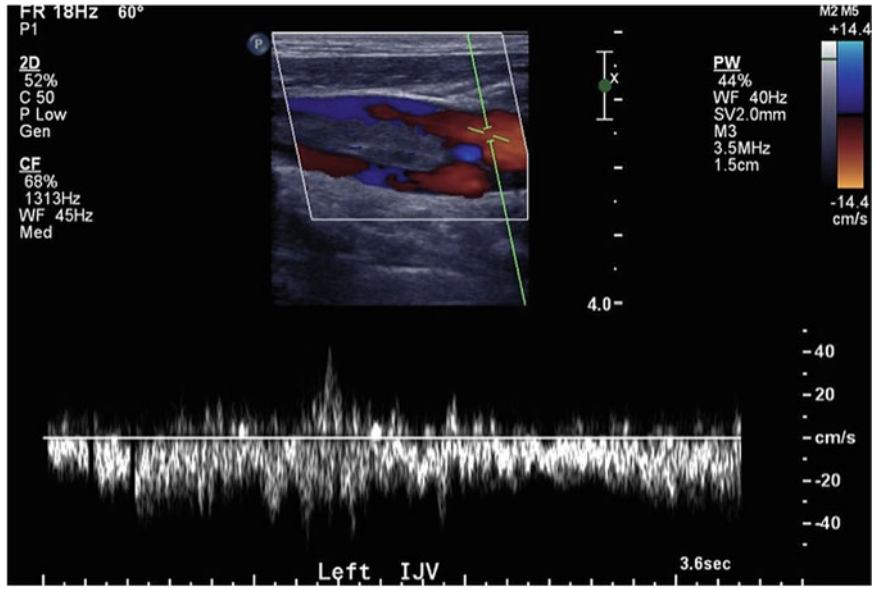

Fig. 13.3 Color Doppler shows partially occlusive thrombus in the internal jugular vein

on duplex ultrasound (Figs. 13.2 and 13.3). Though venography historically has been the gold standard for diagnosing acute venous thrombosis, it is an invasive procedure and thus less preferred (Bettmann 1988; Lensing et al. 1992).

Ultrasound is sensitive and specific for the diagnosis of UEDVT, but false-negative studies may occur due to shadowing from the clavicle that obscures thrombus in the subclavian vein or presence of non-occlusive thrombus that is adherent to the vein wall (Prandoni et al. 1997).

To aid in diagnosis, a clinical prediction score has been developed among patients hospitalized with suspicion of UEDVT. The score incorporates

the presence of indwelling venous device, localized pain, unilateral pitting edema, and presence or absence of an equally likely alternative diagnosis, assigning one point to each item. UEDVT was objectively confirmed with ultrasound, and the prediction score was validated among another group of patients. A score of 2 or 3 identified high-probability patients, with 60–74 % prevalence of UEDVT (Constans et al. 2008).

Prevention

There are no standard guidelines for prevention of CRT. Various non-pharmacologic measures have been studied for the prevention of CRT. Indwelling venous catheters should be placed in appropriate position by experienced clinicians with adequate technological support. Measures to decrease intimal damage include selection of appropriate devices (smallest diameter of catheter with fewest number of lumens), ideal sites of access (right preferred over left, subclavian preferred over other sites), and adequate catheter care (Biffi et al. 2009). Intermittent pneumatic compression devices of the arms theoretically could be beneficial but have not been adequately studied (Berlin et al. 1999; Knight and Dawson 1976). Though earlier studies focusing primarily on oncologic patients demonstrated that various antithrombotic agents are effective in preventing CRT, recent studies have shown no significant benefit (Lee et al. 2006; Abdelkefi et al. 2004; Bern et al. 1990; Eastman et al. 2001; Heaton et al. 2002; Karthaus et al. 2006; Klerk et al. 2003; Magagnoli et al. 2005; Monreal and Davant 2001; Tesselaar et al. 2004). Low-dose warfarin was shown to be effective in the prevention of CRT primarily in oncologic patients with central venous catheters. One mg per day of warfarin compared with no warfarin for 90 days resulted in lower rates of asymptomatic (9.5 % vs. 37.5 %) and symptomatic (9.5 % vs. 32.5 %; $p=0.001$) thrombosis (Bern et al. 1990). In a study on cancer patients undergoing chemotherapy, patients who received anticoagulation with nadroparin or coumarins had significantly lower rates of CRT in chest ports (1 % vs. 33 %; OR, 34.8; 95 % CI, 7.3–165) but not in arm ports (32 % vs. 28 %) (Tesselaar et al. 2004). However, there was no significant difference in patients who received coumarins versus those who did not receive coumarins in a non-randomized study of patients with melanoma or renal cell cancer (Eastman et al. 2001). Patients with hematologic malignancies and Hickman catheter had no significantly different occurrence of CRT when they received 1 mg warfarin per day compared to the control arm (Heaton et al. 2002).

Data for heparins are similarly mixed. In a double-blind placebo-controlled phase III trial in 439 patients undergoing cancer chemotherapy, Karthaus et al. reported no significant difference between dalteparin 5,000 IU and placebo in frequency of CRT (3.7 % vs. 3.4 %; $p=0.88$; RR, 1.0883; 95 % CI, 0.37–3.19) (Karthaus et al. 2006). In a prospective randomized controlled trial in 128 hematology–oncology patients, those who had continuous infusion of unfractionated heparin of 100 U/kg daily had lower risk of CRT than patients receiving 50 mL daily of normal saline (1.5 % vs. 12.6 %; $p=0.03$) (Abdelkefi et al. 2004). Currently available evidence is inconclusive regarding the use of anticoagulation for the prevention of CRT, while the role of antithrombotic prophylaxis in the prevention of CRT in non-oncologic patient populations has not been studied to date. In the face of conflicting data, the American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (Guyatt et al. 2012) suggest against routine prophylaxis with heparin, low-molecular-weight heparin, and vitamin K antagonists.

Though the mechanism is unclear, urokinase instillation into CVCs is effective in reducing CRT in pediatric populations, but it has not been adequately studied in adults (Dillon et al. 2004; Kalmanti et al. 2002).

Treatment of CRT usually consists of anticoagulation with or without instillation of fibrinolytic agent in the catheter lumen. Parenteral anticoagulation with full-dose unfractionated heparin, LMWH, or fondaparinux should be initiated as soon as the CRT is suspected or confirmed. Catheter removal is warranted only if there is catheter malfunction and certain catheter-related

infections (such as *S. aureus*, gram-negative bacilli, and Candida species) or when the catheter is no longer needed. Anticoagulation after removal is suggested for a minimum duration of 3 months if the DVT involves the axillary or more proximal veins (Guyatt et al. 2012). If the catheter is not removed, anticoagulation should be continued as long as the CVC is in place. Once removed, anticoagulation is often continued another 6 weeks to prevent new thrombosis.

Catheter malfunction due to intraluminal thrombus can be treated by a thrombolytic instillation into the catheter lumen (Dillon et al. 2004; Kalmanti et al. 2002). Pharmacological thrombolysis or mechanical thrombectomy may be considered in patients who remain symptomatic despite adequate anticoagulation (Kim et al. 2006; Vik et al. 2009). Although SVC filter is a management option for UEDVT in those with contraindications to anticoagulation, failed anticoagulation with PE despite therapeutic anticoagulation, and complications of anticoagulation, data are sparse on the safety and efficacy of SVC filter and it is not recommended by standard guidelines (Usoh et al. 2009).

Complications

Complications associated with CRT include the post-thrombotic syndrome, PE, catheter-related infection, progression to SVC syndrome, and loss of viable vascular access sites (van Rooden, Rosendaal et al.2004; Verso and Agnelli 2003; Monreal and Davant 2001). 15 to 30 % of patients with UEDVT develop the post-thrombotic syndrome, including such signs and symptoms as chronic limb edema, cyanosis, and pain, but it is unclear how many patients with CRT go on to develop this syndrome, as studies generally have been too small (Kahn et al. 2005; Maki and Ringer 1991; Prandoni et al. 2004). Several studies have shown that persistent catheter-related bloodstream infection may be associated with CRT or development of a fibrin sheath (van Rooden, Molhoek et al.2004; Kuter 2004; Chemaly et al. 2002; Da Costa et al. 2002; Mehall et al. 2002; Ngo and Murphy 2005).

Pulmonary embolism is less common in UEDVT than lower extremity DVT, but the risk is not negligible, ranging from 6 to 36 % after UEDVT (King et al. 2006; Monreal and Davant 2001; Sticherling et al. 2001). In the prospective RIETE registry of patients with symptomatic venous thromboembolism, of patients presenting with pulmonary embolism, just 9 % had UEDVT compared with 29 % who had lower extremity DVT (Munoz et al. 2008).

Clinical Vignette 2

A 79-year-old woman presents to the emergency room with complaints of dyspnea and bilateral arm swelling that have been progressive over the last 6 months. Vital signs are normal. The left arm is more swollen than the right. Electrocardiogram reveals paced rhythm. Chest X-ray shows an implanted pacemaker with leads in the right atrium and ventricle but no abnormalities. Two-dimensional echocardiography shows left ventricular ejection fraction of 30 % and right ventricular dilation and dysfunction. Doppler ultrasound of the upper extremities shows no thrombosis in the axillary veins and more distal veins; the subclavian veins are not visualized. What is the next best step?

1. Right arm cooling.
2. Start therapeutic anticoagulation.
3. Apply compression wraps and extremity elevation.
4. Removal of pacemaker.
5. Venography.

Clotting Related to Implantable Cardiac Devices

Pathophysiology, Epidemiology, and Risk Factors

Venous complications related to implantable cardiac devices include thrombosis, stenosis, and occlusion of the central veins. In patients with

implanted pacemakers, the incidence of device-related venous abnormalities is high, ranging from 20 to 60 % depending on the population studied, though in most cases patients are asymptomatic and the clinical relevance remains uncertain (Antonelli et al. 1989; Crook et al. 1977; Goto et al. 1998; Lickfett et al. 2004; Oginosawa et al. 2002; Stoney et al. 1976).

The pathophysiology is similar to that of CRT. A history of prior transvenous pacing leads and left ventricular ejection fraction less than 40 % are associated with development of venous abnormalities such as thrombosis and stenosis in patients with permanent pacemaker (Da Costa et al. 2002). Dual-coil leads, prior DVT, prior central venous catheter, use of temporary wires, infection, and oral contraceptive agents also increase the risk for cardiac device-related thrombosis in patients with pacemaker or implanted defibrillator (Antonelli et al. 1989; Goto et al. 1998; Lickfett et al. 2004). The number of leads is not a consistent risk factor for device-related thrombosis (Goto et al. 1998). Access site, type of lead (unipolar vs. bipolar), lead material, and lead caliber are not associated with device-related thrombosis (Lickfett et al. 2004; Oginosawa et al. 2002; Stoney et al. 1976).

Clinical Manifestations and Complications

Cardiac device-related thrombosis is usually asymptomatic. In a study looking at 105 patients with implantable defibrillators in place for more than 3 years, a quarter of them had some degree of venous occlusion, but no one was symptomatic (Lickfett et al. 2004). In a smaller study following patients with permanent pacemaker for a year after implantation, 23, more than a third, developed some degree of venous occlusion, but less than 20 % of those patients were symptomatic (Antonelli et al. 1989). When symptomatic, patients with cardiac device-related thrombosis may complain of nonspecific shoulder or neck discomfort or have ipsilateral arm swelling with cyanosis, dilated collateral cutaneous veins at the shoulder or anterior chest wall, and jugular vein distention.

As with CRT, pulmonary embolism, post-thrombotic syndrome, and SVC syndrome may complicate device-related thrombosis (Nishino et al. 1997). A history of prior device-related thrombosis may make lead revision more challenging (Spittell and Hayes 1992).

Diagnosis

Compressive ultrasonography and/or two-dimensional echo combined with pulsed Doppler and color flow evaluation are commonly used to diagnose device-related venous thrombosis (Nishino et al. 1997). Compressive ultrasonography has limitations in diagnosing SVC and innominate venous thrombosis due to shadowing from the clavicle. However, combined color flow and pulse wave Doppler had 94 % sensitivity and 100 % specificity for detecting SVC or innominate vein thrombosis compared with digital subtraction angiography in detecting 19 cases of thrombosis or stenosis in a group of 53 patients (Conte and Orzel 1986).

Spiral computed tomography venography (CTV) and magnetic resonance venography (MRV) are noninvasive and accurate diagnostic tools for detecting deep venous thrombosis; however, the presence of a pacemaker or an implanted defibrillator is a relative contraindication for MR imaging (Spittell and Hayes 1992; Hartnell et al. 1995; Kommareddy et al. 2002; Tello et al. 1993). Conventional venography remains the gold standard and may often be used prior to vascular interventional treatment for thrombosis and to assess the response to the treatment (Bettmann 1988).

Management

There are no universal guidelines for the primary prevention of implantable cardiac device-related thrombosis because of insufficient data. One small study found that warfarin in high-risk patients may be beneficial in preventing device-related venous

complications, but further study is required (Costa et al. 2009).

Device-related venous thrombosis may be treated with a multimodal approach, involving anticoagulation and catheter-directed thrombolytic therapy (depending on the extent of the thrombosis and the severity of the symptoms), followed by a minimum of 3 months of anticoagulation, venous decompression as needed, and balloon angioplasty with stenting for treatment of residual stricture (Chan et al. 2002; Montgomery et al. 1985; Spittell et al. 1990).

Anticoagulation is the cornerstone of therapy in patients with symptomatic device-related thrombosis. Thrombolytic therapy used in cases of lower extremity venous thrombosis may improve early patency or recanalization, but this has not been studied for symptomatic cardiac device-related thrombosis. Furthermore, many patients may not be candidates for thrombolysis due to bleeding risk and other clinical factors.

At times, percutaneous venoplasty may be performed to treat venous stenosis or occlusion and/or to facilitate pacemaker revision (Chan et al. 2002; Montgomery et al. 1985; Spittell et al. 1990). Surgical treatment options, though associated with significant morbidity, exist for the management of the lead-related thrombosis that is not amenable to anticoagulation and endovascular interventional treatment options (Barakat et al. 2000).

Clinical Vignettes

Clinical Vignette 1 answer: 2. The patient has catheter-related right UEDVT. He was treated with anticoagulation for a total of 3 months, and the PICC was initially left in place. Antibiotics were continued, and the catheter was removed at the completion of the antibiotic treatment.

Clinical Vignette 2 answer: 5. Venography showed visible thrombus or obstruction in the left subclavian vein and formation of collaterals. The pacemaker was left in place, and the patient was treated with anticoagulation for 3 months.

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Heparin-Induced Thrombocytopenia

14

John R. Bartholomew

List of Abbreviations

ACCP	American College of Chest Physicians
ACS	Acute coronary syndrome
ACT	Activated clotting time
aPTT	Activated partial thromboplastin time
CABG	Coronary artery bypass grafting
DIC	Disseminated intravascular coagulation
DTI	Direct thrombin inhibitor
DVT	Deep vein thrombosis
FDA	Food and Drug Administration
HAT	Heparin-associated thrombosis
HEP	Heparin expert probability score
HISN	Heparin-induced skin necrosis
HIPA	Heparin-induced platelet aggregation
INR	International normalized ratio
ITP	Idiopathic thrombocytopenia purpura
HIT	Heparin-induced thrombocytopenia
LMWH	Low-molecular-weight heparin
MI	Myocardial infarction
OD	Optical density
PCI	Percutaneous coronary intervention
PE	Pulmonary embolism
PF4	Platelet factor 4
PT	Prothrombin time
PTA	Percutaneous transluminal angioplasty
PTCA	Percutaneous transluminal coronary angiography
SRA	Serotonin release assay
TF	Tissue factor
TTP	Thrombotic thrombocytopenia purpura
UFH	Unfractionated heparin
VKA	Vitamin K antagonist
VLG	Venous limb gangrene
WISN	Warfarin-induced skin necrosis

Clinical Vignette 1

A 65-year-old female recently had open heart surgery. The procedure and postoperative hospital course were uneventful, and she was discharged home on day 5 following surgery. One week later she presented to the emergency room with complaints of shortness of breath and chest pain. A chest X-ray revealed a small pleural effusion, and an electrocardiogram was unremarkable except for sinus tachycardia. A routine complete blood count demonstrated mild thrombocytopenia (134,000/μl). A computed axial tomography (CAT scan) of the chest revealed right and left lobar pulmonary emboli. The emergency room doctor initiates intravenous UFH, and you are consulted shortly after admission for worsening thrombocytopenia.

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Introduction

Heparin-induced thrombocytopenia (HIT) is a transient, prothrombotic immune-mediated complication of UFH or low-molecular-weight heparin (LMWH) that can result in venous or arterial thrombosis, amputation, or death. It most commonly presents within 5–14 days of administration in a patient with no recent heparin exposure and is referred to as typical-onset HIT. However, HIT may also develop within a few hours or days if there was a recent exposure, a condition known as rapid-onset HIT, or can occur several days or even weeks after either UFH or LMWH has been discontinued, a form known as delayed-onset HIT.

Thrombocytopenia, the most common presentation of HIT once considered necessary for the diagnosis, is no longer considered essential as a 50 % reduction in the platelet count from the patient baseline count is now deemed a more specific finding. Deep vein thrombosis (DVT) and pulmonary embolism (PE) are the most common venous thrombotic events, while the most frequent arterial thrombosis is acute limb ischemia. The diagnosis relies on both clinical findings and laboratory detection of HIT antibodies. Immediate cessation of UFH or LMWH is recommended once the diagnosis is suspected, and a non-heparin anticoagulant to prevent and/or treat the potentially devastating complications should be initiated without delay.

A growing concern is overdiagnosis and overtreatment of HIT. This is largely felt due to the challenge of correctly diagnosing HIT because thrombocytopenia is common in hospitalized patients, UFH or LMWH administration is frequently used, and of the fear among clinicians of missing the diagnosis (Cuker and Cines 2012; Cuker et al. 2010).

Incidence

HIT occurs in approximately 1–5 % of all patients exposed to UFH but in less than 1 % of individuals receiving LMWH, although LMWH may account for a growing number of HIT cases because of its increasing use in clinical practice (Cuker and Cines 2012; Cuker et al. 2010; Shantsila et al. 2009; Warkentin et al. 2006; Martel et al. 2005). The incidence of HIT also varies depending on the type of UFH preparation (bovine lung > porcine intestine); longer duration of exposure (up to day 14); patient population (women > men); age (middle-aged and older patients > young adults and children); trauma; surgical (especially orthopedic and cardiac) > medical > obstetric patients); and dose of the anticoagulant (treatment > prophylaxis) (Cuker and Cines 2012; Warkentin 2003; Warkentin et al. 1995; Prandoni et al. 2005). In a meta-analysis of predominantly surgical patients receiving UFH or LMWH for thromboprophylaxis, the risk for HIT was reported as 2.6 and 0.2 %, respectively (Martel et al. 2005). In the cardiac surgery populations it occurs in 1–3 %, whereas in intensive care units it is reported to be less common (0.4–1 %), and in the obstetric population it is less than 0.1 % (Prandoni et al. 2005; Crowther et al. 2010; Fausett et al. 2001).

HIT antibodies are found much more often and are detected in approximately 20 % of patients treated with UFH and 8 % of those receiving LMWH (Shantsila et al. 2009; Amiral et al. 1996). This rate is also dependent on the clinical situation. According to Amiral et al., as many as 20 % of orthopedic patients will develop heparin antibodies, but the numbers are much higher in the patient who undergoes cardiopulmonary bypass surgery approaching 50–70 % (Shantsila et al. 2009; Amiral et al. 1996).

Pathogenesis

HIT is an adverse immune-mediated disorder that develops following exposure to UFH or LMWH. It is less common with LMWH due to differences in the stoichiometry of the heparin/PF4 complex, but either agent can release platelet factor 4 (PF4), a heparin-neutralizing protein found in the alpha granules of platelets (Martel et al. 2005; Linkins et al. 2012). In susceptible individuals, an immune response develops resulting in the formation of platelet-activating

antibodies, usually IgG immunoglobulins. These antibodies (also referred to as "HIT antibodies") recognize multimolecular complexes of PF4 and heparin that form on the FcγIIa receptors of the platelet surface. This results in the formation of immune complexes that can result in platelet activation and the release of procoagulant platelet-derived microparticles that promote thrombin formation. These immune complexes are also capable of binding to heparin sulfate on endothelial cells and monocytes and initiate the release of tissue factor (TF) which further contributes to the prothrombotic state of HIT (Cines et al. 2007; Warkentin 2006; Kelton 2005; Bartholomew et al. 2005).

Clinical Features

The major clinical features of HIT are thrombocytopenia and thrombosis. Thrombocytopenia develops in as many as 85–90 % of patients. It is normally defined as a platelet count less than 150,000 mm^3 (Shantsila et al. 2009; Warkentin 2003). However, in a report of 142 serologically confirmed cases the median platelet count was much lower (59,000/μl) and platelet counts less than 15,000/μl were reported (Warkentin 1998).

Not all HIT patients develop thrombocytopenia, and platelet counts greater than 500,000/μl have been reported (Warkentin 1998). Further, a 50 % or greater drop in the platelet count from the patients' baseline in patients receiving either UFH or LMWH has been found to be a more sensitive predictor for its diagnosis (Warkentin 2003; Warkentin et al. 2003; Warkentin 1998; Warkentin 2008; Arepally and Ortel 2006). Potentially confusing the diagnosis of HIT further for clinicians, up to 25 % of patients will develop thrombosis before the onset of thrombocytopenia (Warkentin et al. 1995; Greinacher et al. 2005). Despite thrombocytopenia, bleeding is uncommon in patients with HIT.

More than half of all patients with HIT will experience DVT or PE, whereas fewer (approximately 10 %) will suffer an arterial event. Amputation rates of 10–21 % and mortality rates of between 5 to 10 % and as high as 17 to 30 % have been reported (Warkentin 2003; Arepally and Ortel 2006; King and Kelton 1984; Warkentin and Kelton 1996; Jang and Hursting 2005).

It is also well recognized that thrombocytopenia can develop without clinical signs or symptoms of thrombosis, a condition referred to as "isolated HIT." These individuals have a higher incidence of thrombosis if left untreated, reported to be between 17 and 55 % (Warkentin and Kelton 1996; Wallis et al. 1999; Zwicker et al. 2004).

Although thrombosis was originally reported to occur predominately in the arterial circulation, it is now well accepted that the majority of patients will develop DVT of the lower extremity and/or PE (Warkentin 2003). DVT is also found in the upper extremity where it is usually associated with central venous catheter or pacemaker wire placement. Other less frequently reported venous events include mesenteric vein thrombosis, adrenal hemorrhagic infarction with adrenal vein thrombosis, and cerebral vein thrombosis.

The most common arterial thrombosis is acute limb occlusion. This most often develops in patients with arteriosclerosis or at the site of a recent endovascular or surgical procedure. Heparin-induced thrombocytopenia may also present as an acute thrombotic stroke, myocardial infarction (MI), intracardiac thrombus, thrombosis of an extracorporeal circuit, or mesenteric or renal artery thrombosis. Occlusion of saphenous vein and less commonly arterial bypass grafts have also been reported following open heart surgery (Liu et al. 2002).

Several atypical manifestations of HIT including warfarin-induced venous limb gangrene (VLG), warfarin-induced skin necrosis (WISN), heparin-induced skin necrosis (HISN) (Fig. 14.1), transient global amnesia, disseminated intravascular coagulation (DIC), and an acute systemic (anaphylactoid) reaction following intravenous bolus of UFH have all been recognized (Warkentin et al. 1997; Srinivasan et al. 2004).

Warfarin-induced VLG is characterized by distal extremity necrosis, an ipsilateral limb DVT, and a supratherapeutic international normalized ratio (INR). It differs from WISN which is more commonly found in areas of fatty tissue, especially the breasts, buttocks, or thighs

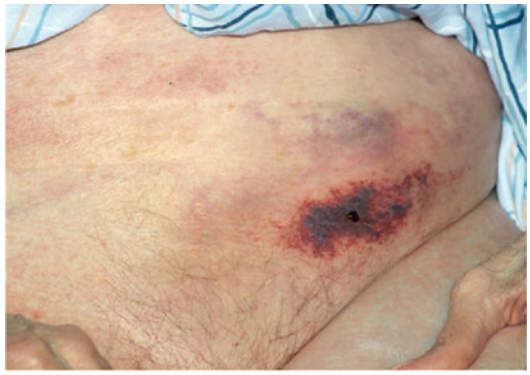

Fig. 14.1 Heparin-induced skin necrosis

(Warkentin et al. 1997; Srinivasan et al. 2004). Either condition may develop when a vitamin K antagonist (VKA) such as warfarin is initiated during acute HIT prior to the patients' clinical improvement and/or platelet count recovery. In this situation, rapid depletion of the vitamin K-dependent natural anticoagulant protein C results, creating an additional prothrombotic condition that combined with the preexisting hypercoagulable state of HIT leads to increased thrombosis. Both forms of warfarin-induced necrosis can also develop when a VKA is administered unopposed (without the addition of a direct thrombin inhibitor (DTI) (Warkentin et al. 1997; Srinivasan et al. 2004). WISN is 100 times more likely in patients with HIT (5–10 %) compared to non-HIT populations (0.001 %) (Warkentin 2006).

Patients can also develop heparin-induced skin lesions that range from erythematous plaques and nodules to overt skin necrosis. Heparin-induced skin necrosis most often occurs at the site of a subcutaneous injection of UFH or LMWH but has been reported distal to the injection. The necrotic lesions are extremely painful and characterized by an area of central ischemia surrounded by erythema (Warkentin 2007).

An acute systemic reaction developing approximately 5–30 min following an intravenous bolus of UFH or up to 1 h after subcutaneous injections of LMWH has also been described (Mims et al. 2004). It is characterized by an abrupt, transient fall in the platelet count as well as fever, chills, tachycardia, hypertension, dyspnea, chest pain, and diarrhea. Transient global amnesia and sudden cardiorespiratory collapse and death have also been reported (Warkentin, Greinacher et al. 2008).

DIC is an uncommon finding in HIT but is characterized by hypofibrinogenemia, a transient acquired deficiency of antithrombin and protein C and a prolonged INR and activated partial thromboplastin time (aPTT). Schistocytes, livedo reticularis, renal failure, and other signs of microvascular thrombosis may also be present.

Temporal Patterns of HIT

There are three distinct temporal patterns of HIT: typical onset, rapid onset, and delayed onset (Warkentin and Kelton 2001). Typical-onset HIT occurs in the majority of patients, developing within 5–14 days after a first exposure to either UFH or LMWH.

Rapid-onset HIT can occur within hours to days after either anticoagulant is initiated. In a series of 243 serologically confirmed cases, 30 % of individuals developed this form. The median time to onset of thrombocytopenia was 10.5 h, and all patients had received UFH or LMWH within the preceding 100 days and most within 30 days (Warkentin and Kelton 2001). Rapid-onset HIT results from residual circulating heparin–PF4 antibodies that developed during a recent exposure and is not an anamnestic immune response.

Delayed-onset HIT usually develops within 7 to as many as 40 days after either UFH or LMWH is discontinued, often after the patient has been discharged. Patients are found to have very high titers of HIT antibodies and are generally recognized because of the development of a new venous or arterial thrombosis and a low platelet count that worsens if UFH is administered. *Clinical vignette number 1 listed above is an example of delayed-onset HIT*. The patient developed an acute PE 1 week after hospital discharge (off of all anticoagulants) following open-heart surgery where she had recently been exposed to UFH. She had only mild thrombocytopenia on

presentation to the emergency room that worsened after UFH was initiated. The drop in her platelet count when re-exposed to UFH and the new thrombosis were the keys to the diagnosis.

Delayed-onset HIT may represent only about 3–5 % of all HIT cases (Warkentin, Greinacher et al. 2008). According to Warkentin, this form should be differentiated from a delayed recognition of HIT in patients for whom the platelet count was not closely followed or the diagnosis not considered (Fausett et al. 2001; Warkentin, Greinacher et al. 2008). Patients with delayed-onset HIT develop thrombocytopenia that begins or worsens after stopping UFH and may be thrombocytopenic when they present with a new thrombosis. If UFH is given, their platelet count will drop further (Warkentin 2011).

Diagnosis of HIT

HIT should be considered in any patient who develops thrombocytopenia while receiving UFH or LMWH. Psuedothrombocytopenia, sepsis, thrombotic thrombocytopenic purpura (TTP), idiopathic thrombocytopenic purpura (ITP), posttransfusion purpura (PTP), alcohol related, aplastic anemia, hypersplenism, DIC, drug induced (especially glycoprotein IIb/IIIa inhibitors and thienopyridines), mechanically induced by an intra-aortic balloon pump, and hemodilution from blood product transfusions must all be considered in the differential diagnosis.

Warkentin introduced the concept of "pseudo-heparin-induced thrombocytopenia," defined as conditions that may mimic HIT and may also cause thrombocytopenia and thrombosis (Table 14.1).

Although thrombosis can be a complication of the patient's age, hospitalization, inactivity, recent surgery, or another underlying hypercoagulable condition, the development of thrombocytopenia or a 50 % drop in the patient's platelet count or new thrombosis or an extension of an existing thrombus while a patient is receiving either UFH or LMWH should alert the clinician to the possibility of HIT. HIT must also be considered in patients resistant to UFH, defined as those individuals who require unusually high doses to attain therapeutic aPTT levels.

Table 14.1 The concept of pseudo-heparin-induced thrombocytopenia—"Conditions that may mimic HIT" (Warkentin and Cuker 2013)

1. Adenocarcinoma
2. Diabetic ketoacidosis
3. Antiphospholipid syndrome
4. Septicemia-associated purpura fulminans
5. Infective endocarditis
6. Paroxysmal nocturnal hemoglobinuria
7. Postsurgical TTP

The diagnosis of HIT is based on both the clinical presentation and laboratory testing. Warkentin and Heddle developed a scoring system to assess the pretest probability of HIT, known as the 4Ts (Warkentin and Heddle 2003). This approach has been helpful in deciding which patient will require further laboratory testing (Warkentin 2003; Warkentin, Greinacher et al. 2008; Warkentin and Heddle 2003; Lo et al. 2006). The test was based on four criteria: thrombocytopenia, timing of the platelet count fall, thrombosis, and exclusion of other causes for thrombocytopenia. Each feature was given a score of 0, 1, or 2 points. Scores of 0–3, 4–5, and 6–8 correspond to a low, intermediate, or high pretest probability (Table 14.2) (Warkentin and Heddle 2003).

The performance of the 4Ts has been evaluated and compared to the serotonin release assay (SRA), generally considered the gold standard for the diagnosis of HIT. Based on the results, the negative predictive value of a low probability score of <3 essentially excludes the diagnosis of HIT, whereas almost half of patients tested with a score of 6–8 will have the diagnosis confirmed (Lo et al. 2006). A new version of the 4T score is currently undergoing prospective evaluation (Warkentin and Linkins 2010). It includes several different characteristics including surgery within the previous 3 days, recurrent venous thrombosis in a patient receiving therapeutic anticoagulation, sepsis without a proven microbial source, and thrombocytopenia associated with initiation of a ventilator (Warkentin and Linkins 2010).

Table 14.2 Estimating the pretest probability of HIT: The four Ts score

4Ts category	2 points	1 point	0 points
Thrombocytopenia Compare the highest platelet count within the sequence of declining platelet counts to determine the % of platelet fall *(select only one option)*	Platelet count fall of >50 % AND nadir of ≥20,000 mm^3 AND no surgery within preceding 3 days	Platelet count fall of >50 % BUT surgery within preceding 3 days OR Any combination of platelet fall and platelet nadir that does not fit criteria for 2 point score or 0 point score (e.g., fall in platelets of 30–50 % or nadir of 10–19,000 mm^3	Platelet count fall <30 % or Any platelet nadir <10,000 mm^3
Timing of platelet count drop or thrombosis Day 0 = first day of most recent heparin exposure *(select only one option)*	Onset between days 5 and 10 after starting UFH Or within 1 day of starting UFH and recent exposure to UFH within 5–30 days	Consistent with platelet count fall of 5–10 days but not clear (missing counts) Platelet fall within 1 day of starting heparin AND exposure within the past 31–100 days Platelet count fall after day 10	Platelet count falls too early (≤ 4 days) without heparin exposure in the past 100 days
Thrombosis or other clinical sequelae (select only one option)	Confirmed new thrombosis (venous or arterial) Skin necrosis at injection site Acute systemic reaction to IV UFH bolus Adrenal hemorrhage	Progressive or recurrent thrombosis in a patient receiving therapeutic anticoagulants Erythematous skin lesions at UFH injection sites Suspected thrombosis (confirmation pending with imaging)	Thrombosis suspected
Other causes of thrombocytopenia (select only one option)	No alternative explanation for platelet fall evident	*Possible other causes evident:* Sepsis without proven microbial source Thrombocytopenia associated with initiation of ventilator Others	Probable other causes present: Within 72 h of surgery Confirmed bacteremia/fungemia Chemotherapy or radiation within past 20 days DIC due to non-HIT cause Posttransfusion purpura (PTP) Platelet count <20,000 mm^3 AND given a drug implicated in causing thrombocytopenia Non-necrotizing skin lesions at LMWH injection site Others

Pretest probability: 6–8 = high, 4–5 = intermediate, 0–3 = low
Adapted from Warkentin and Linkins

There are other pretest scoring systems available. The HIT expert probability (HEP) score is based on the opinions of 26 HIT experts (Cuker et al. 2010). Eight clinical features are evaluated including the magnitude and timing of the fall in platelet count, nadir platelet count, thrombosis, skin necrosis, an acute systemic reaction, bleeding, as well as considerations for other causes of thrombocytopenia. According to Cuker et al. the HEP score demonstrates a high degree of inter-observer agreement and correlates with the results of the HIT laboratory testing. This has led the authors to suggest that using this score may allow clinicians to more confidently reduce the use of alternative anticoagulants in patients suspected HIT (Cuker et al. 2010). The HEP study has not yet been prospectively evaluated (Cuker et al. 2010).

A diagnostic score has also been developed for patients suspected of HIT following cardiopulmonary bypass (CPB) (Lillo-Le-Louet 2004). This method utilizes the platelet count increase after heparin withdrawal from surgery, detection of heparin-dependent antibodies, and absence of other clear causes for thrombocytopenia. The authors found that the development of a biphasic platelet count following CPB (to the first day of suspected HIT), an interval of ≥5 days from CPB to the first day of suspected HIT, and a CPB duration of ≤118 min were independent risk factors for HIT (Lillo-Le-Louet 2004).

Two types of laboratory tests are available: functional tests which detect heparin-dependent platelet activation in the presence of the patient's sera and UFH or LMWH, and antigen assays or immunoassays which measure IgG, IgM, or IgA antibodies that bind PF4 to either anticoagulant. Both tests have high negative predictive values but just moderate positive predictive values. To avoid overdiagnosis of HIT, these tests should only be ordered when there is a moderate to high clinical suspicion for this condition (Warkentin 2006; Warkentin, Greinacher et al. 2008; Warkentin, Sheppard et al. 2005).

Functional assays have greater specificity but are less sensitive. Several tests are available: a heparin-induced platelet aggregation assay (HIPA) and SRA. The HIPA uses platelet-rich plasma mixed with the patient's plasma plus heparin (Warkentin and Heddle 2003). The SRA uses donor platelets labeled with radioactive ^{14}C serotonin and is more sensitive and specific and the standard by which other tests are judged. It is not readily available at all hospitals because it requires radioisotopes and is technically more demanding to perform.

The most commonly used antigen assays are the enzyme-linked immunosorbent assays (ELISA) that are much easier to perform than functional tests and have a higher sensitivity but lower specificity. The results are reported as optical density (OD) readings with any value greater than 0.40 OD considered positive. Patients with higher titers (greater than >1.0 OD) appear more likely to develop thrombosis (Zwicker et al. 2004). A recent single-center study registry evaluated 318 patients with clinically suspected HIT and also found that higher levels of anti-PF4/heparin antibody were associated with increased risk of venous and arterial thrombosis (Baroletti et al. 2012). Unfortunately, false-positive results occur because most of the commercially available immunoassays detect all three immunoglobulin classes, and IgM and IgA do not cause HIT. In addition, not all patients with IgG heparin antibodies will develop HIT; therefore, laboratory results must be considered with the clinical presentation prior to confirming the diagnosis (Warkentin and Sheppard 2006; Warkentin, Sheppard et al. 2005).

Once HIT is suspected, an immunoassay and functional assay should be performed (Cuker and Cines 2012). If both tests are positive the diagnosis of HIT is confirmed, whereas if both are negative, HIT is unlikely. If one test is positive and the other negative, HIT may be considered indeterminate and repeating the tests may be helpful (Arepally and Ortel 2006). It must be remembered that HIT remains a clinical diagnosis and the results of laboratory testing do not always coincide with the clinical picture. A recently published algorithm by Cuker and Cines provides a reasonable working approach for the diagnosis of HIT (Fig. 14.2) (Cuker and Cines 2012; Cuker et al. 2010).

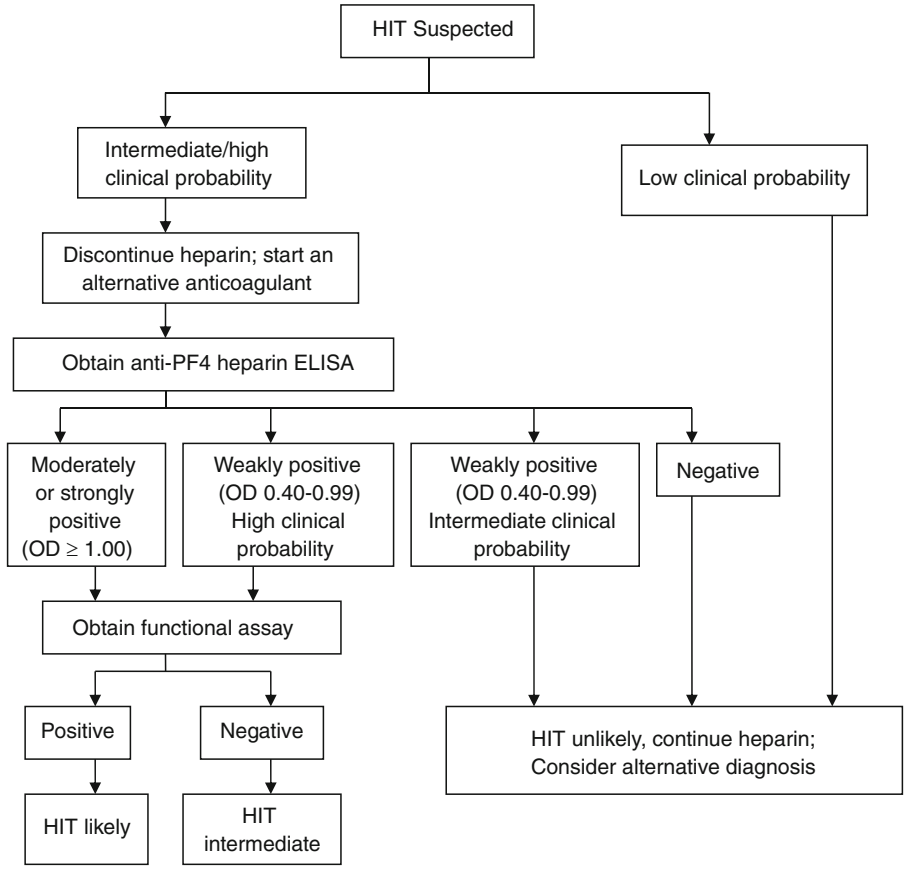

Fig. 14.2 An approach to the diagnosis of patients with suspected HIT. Adapted from Cuker and Cines

Management of HIT

All forms of UFH or LMWH must be discontinued immediately once HIT is suspected, including any found in unsuspected locations; bound to UFH-coated catheters; in arterial line flushes; added to intravenous solutions for angiographic procedures; and administered during dialysis or added to total parenteral nutrition solutions. LMWH should not be substituted for UFH because there is cross-reactivity of the HIT antibodies (Warkentin, Sheppard et al. 2005).

Simply discontinuing UFH or LMWH is inadequate, even without evidence for acute thrombosis. Numerous studies have demonstrated an increased risk for new thrombosis, amputation, or death if an alternative anticoagulant is not initiated (Warkentin, Greinacher et al. 2008; Greinacher et al. 2000; Lubenow et al. 2005; Lewis et al. 2001; Hirsh et al. 2004). Treatment should not be delayed while awaiting laboratory confirmation as this only increases the risk for further complications (Greinacher et al. 2000; Lubenow et al. 2005). Several additional general recommendations are advised: avoiding platelet transfusions unless there is active bleeding (seldom observed despite thrombocytopenia), delaying administration of a VKA until the platelet count has recovered to ≥150,000/μl, and avoiding placement of vena caval filters (Warkentin, Greinacher et al. 2008; Warkentin and Cuker 2013) (Table 14.3).

Direct Thrombin Inhibitors

There are two DTIs approved by the Food and Drug Administration (FDA) for the treatment of HIT, argatroban and lepirudin; one for prevention of isolated HIT, argatroban; and two for patients with HIT requiring PCI, argatroban and bivalirudin (Fig. 14.3).

Table 14.3 Treatment guidelines for HIT

1. Discontinue heparin or low-molecular-weight heparin immediately once HIT is suspected.
2. Remove any hidden source(s) of heparin or LMWH.
3. Do not wait for laboratory confirmation to begin treatment.
4. Initiate an alternative anticoagulant using a direct thrombin inhibitor.
5. Do not start warfarin until the platelet count has recovered to at least 150,000 mm.^{3}
6. Begin with low doses of warfarin (no higher than 5 mg) and overlap with a DTI a minimum of 5 days and until the INR is ≥2.0 for 2 consecutive days.
7. Avoid placement of inferior vena caval filters.
8. Avoid platelet transfusions unless the patient is actively bleeding.

Lepirudin

Lepirudin is a recombinant form of hirudin, the natural anticoagulant derived from the medicinal leech, *Hirudo medicinalis*. It irreversibly binds thrombin, is eliminated by the kidneys, and has a half-life of approximately 1.3 h. Lepirudin is given intravenously, although non-FDA-approved subcutaneous use has been reported.

In three prospective trials referred to as heparin-associated thrombocytopenia or HAT-1, -2, and -3, 403 treated patients were compared to 120 historical controls who were given the best available therapy at the time of the study (Greinacher et al. 2000; Lubenow et al. 2005). Serological confirmation was required before lepirudin was initiated. The combined outcomes of new thrombosis, amputation, and death were lower among patients receiving lepirudin when compared to controls. Further analysis of the outcomes, however, only demonstrated a statistical difference in new thromboembolic events but not amputations or death. Bleeding was also significantly higher in the lepirudin groups compared to the historical controls (Arepally and Ortel 2006; Greinacher et al. 2000; Lubenow et al. 2005).

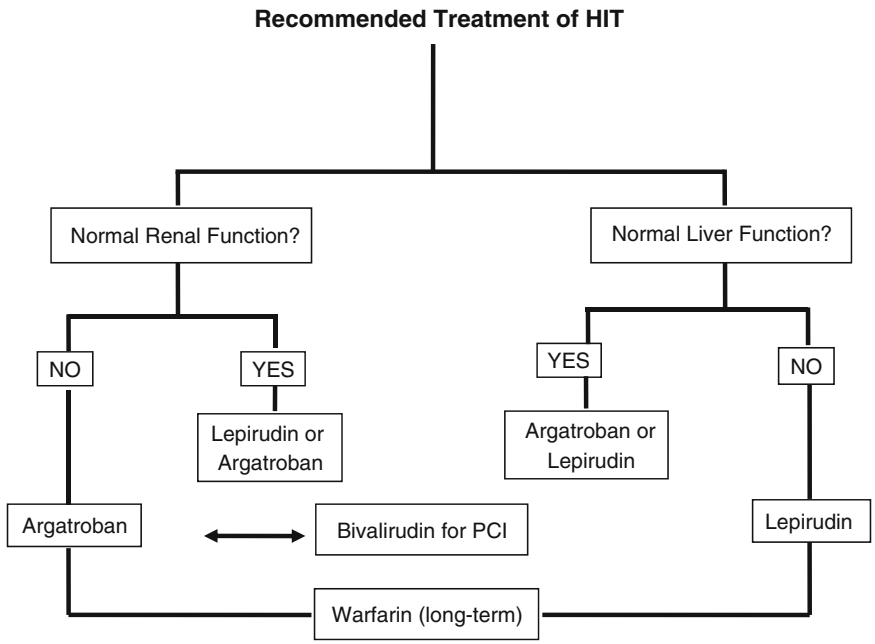

Fig. 14.3 Recommended treatment of HIT

In the initial HAT trials, patients with normal renal function received a weight-based bolus of 0.4 mg/kg followed by an infusion of 0.15 mg/kg/h. Based on data from the pooled analysis of the HAT trials, the manufacturers no longer recommend a bolus dose unless there is perceived life- or limb-threatening thrombosis (Cuker and Cines 2012; Linkins et al. 2012; Warkentin, Greinacher et al. 2008). In that situation, they recommend lepirudin be given at a lower bolus dose of 0.2 mg/kg. They also advise lowering the initial infusion rate to between 0.005 and 0.10 mg/kg/h depending on the renal function (Cuker and Cines 2012; Greinacher et al. 2000; Lubenow et al. 2005; Tardy et al. 2006).

Lepirudin is monitored using the aPTT (target to 1.5–2.5 times the baseline level) and should be checked 4 h after initiating therapy, with any dose adjustments, and daily once the patient is therapeutic.

Lepirudin lacks cross-reactivity with UFH or LMWH, but anti-hirudin antibodies develop in as many as 60 % of all patients (Cuker et al. 2010; Warkentin 2003; Song et al. 1999). These antibodies are not associated with an increased risk for thrombosis but may extend lepirudin's half-life and thus require more frequent dose adjustments. There have also been accounts of anaphylaxis and death in patients re-exposed to lepirudin; therefore, caution is advised if using it more than once (Arepally and Ortel 2006; Greinacher, Lubenow et al. 2003). The manufacturer of lepirudin discontinued its production and distribution as of April 2012 (Cuker and Cines 2012).

Argatroban

Argatroban is a small synthetic molecule derived from l-arginine. It binds in a reversible fashion to the catalytic site of thrombin, is eliminated via hepatobiliary excretion, and has a half-life of 39–51 min. Argatroban lacks cross-reactivity with UFH and is currently approved for prevention in cases of isolated HIT, for treatment of HIT, and in patients requiring PCI.

Two prospective trials (Arg 911 and Arg 915) involving 772 patients with HIT or suspected HIT were compared to historical controls. The combined outcomes of death, amputation, and new thrombosis were lower among individuals receiving argatroban compared to controls (34 % versus 43 %) (Lewis et al. 2001; Hirsh et al. 2004; Lewis et al. 2003). Similar to the HAT trial results (with lepirudin), fewer new thromboembolic events were identified with argatroban and there were no differences in the rates of amputation. The argatroban trial results differed, however, from the HAT trials, as death due to thrombosis was significantly reduced and there was no increase in bleeding compared to historical controls. Unlike the lepirudin trials where anticoagulation was not started until there was serological confirmation of HIT, argatroban was initiated before those tests were reported. Consequently, 36 % of the enrollees were found to be antibody negative on post hoc testing (Cuker and Cines 2012).

No loading dose is required, and initiation using a continuous infusion of 2 μg/kg/min is recommended. Lower doses of argatroban starting with doses between 0.5 and 1.2 μg/kg/min are advised for patients with heart failure, severe anasarca, post-cardiac surgery, moderate to severe liver disease, and multisystem organ failure (Begelman et al. 2008; Hursting and Soffer 2009).

Argatroban is monitored using the aPTT targeted to 1.5–3.0 times the baseline level. The aPTT should be evaluated 2 h after initiating treatment and with dose adjustments. It should be checked daily once therapeutic levels are attained. No antibody formation has been reported with argatroban.

Bivalirudin

Bivalirudin is a small synthetic 20-amino-acid peptide that is a specific and reversible inhibitor of thrombin. Currently, the major indications for bivalirudin are for use in patients with unstable angina undergoing PTCA and also with provisional glycoprotein IIb/IIIa receptor inhibitor treatment to reduce acute ischemic events in select patients undergoing PCI.

Bivalirudin is approved by the FDA for PCI in patients who have or are at the risk for HIT (Mahaffey et al. 2003). An infusion bolus of 0.75 mg/kg is recommended followed by a 1.75 mg/kg/h infusion over 4 h to attain a target ACT of >300 s. Bivalirudin has also been used "off label" for the treatment of HIT patients in a number of small studies (Chamberlin et al. 1995; Bartholomew 2007). It has several advantages including a shorter half-life (25 min), mostly enzymic (80 %) and minimal renal (20 %) metabolism, low immunogenicity, and a minimal effect on the INR (Bartholomew 2007).

Bivalirudin has been used extensively in patients with ACS and also as an alternative anticoagulant to UFH in trials involving patients with HIT during on-pump or off-pump cardiac surgery (Dyke et al. 2007; Koster et al. 2007).

Alternative Therapies

Fondaparinux

Fondaparinux is a synthetic pentasaccharide metabolized by the kidneys and has a half-life of 17–21 h. It is a selective indirect factor Xa inhibitor that binds specifically to antithrombin and inhibits thrombin generation. It does not prolong the prothrombin time (PT), INR, or aPTT, and laboratory monitoring is usually not required.

Clinical Vignette 2

A 61-year-old male underwent left total knee arthroplasty. Thromboprophylaxis was initiated with fondaparinux 2.5 mg subcutaneously daily, and he was discharged home on day 3 postoperatively. On day 10 following surgery, he presented to an emergency room with sudden onset of shortness of breath and chest pain. He was tachycardic and had lower extremity swelling. A computed tomography pulmonary arteriogram (CTPA) revealed bilateral PE, and a venous ultrasound demonstrated a left popliteal DVT. His platelet count had declined from a baseline at the time of surgery of 193,000 to 74,000/μl. You are consulted to see this patient.

Although there was no documented use of UFH or LMWH during his hospital stay (or previously), he was immediately started on the DTI and argatroban, and both an ELISA (anti-PF4/heparin antibody) and SRA were obtained. Both test results were positive with an OD of 3.611 and 100 % inhibition for the anti-PF4/heparin antibody. While on argatroban, his platelet count began to rise, and once recovered, he was converted to warfarin.

Fondaparinux is approved for the prophylaxis and treatment of DVT and PE. It has been used "off label" in a number of patients with HIT (Efird and Kockler 2006). Warkentin et al. reported that immunogenicity was similar between fondaparinux and LMWH in studies involving 2,726 patients. They found that PF4/fondaparinux was poorly recognized by HIT antibodies and believed that the risk of developing HIT was very low (Warkentin, Cook et al. 2005). Subsequently, Warkentin and others have published several case reports of HIT-associated fondaparinux and suggest that it can cause a similar disorder resembling HIT on rare occasions (Efird and Kockler 2006; Warkentin et al. 2007; Warkentin 2008; Warkentin et al. 2012; Warkentin, Cook et al. 2005; Savi et al. 2005). Some of these reports have other potential causes for thrombocytopenia and/or had inadequate testing to confirm the diagnosis of fondaparinux-associated HIT. Many experts dispute its existence.

Fondaparinux has been successfully utilized in the treatment of HIT as an alternative to the more expensive DTIs which require frequent monitoring. The latest ACCP guidelines suggest it be used in patients with a remote history of HIT and in pregnant patients with acute or subacute HIT if danaparoid is not available (Linkins et al. 2012; Warkentin et al. 2011). Despite this

recommendation, physicians should be alert to this possible side effect when treating patients with fondaparinux.

Desirudin

There is little data on the use of desirudin, a subcutaneously administered DTI currently approved by the FDA for DVT prophylaxis in patients undergoing hip replacement surgery. It is a bivalent DTI with moderate thrombin-binding affinity. In the PREVENT-HIT study, 16 patients were enrolled in an open-label, multicenter study designed to evaluate a twice-daily fixed dose of desirudin (15 or 30 mg) compared to argatroban. There were no major bleeding events or new thrombosis in the desirudin arm compared to one case of worsening of an existing thrombosis and two major bleeds in the argatroban arm (Boyce et al. 2011). In addition, the average medication cost per course was substantially lower in patients receiving desirudin (Boyce et al. 2011).

Danaparoid

Danaparoid is a non-heparin low-molecular-weight glycosaminoglycan derived from porcine intestinal mucosa. It is recommended for the treatment and prevention of HIT-associated thrombosis by the ACCP but is no longer available in the United States.

None of the above available alternative anticoagulants have an antidote. If clinically significant bleeding occurs, the agent should be discontinued immediately and supportive therapy initiated.

Warfarin

Warfarin should be avoided in patients with acute HIT to avoid warfarin-induced VLG or skin necrosis. The most recent ACCP guidelines and numerous experts advise that warfarin should be started only after the patient has improved clinically and platelet count has recovered to >150,000/μl; warfarin is initiated in lower doses (≤5mgs) and overlapped concurrently with a DTI for a minimum of five days; and DTI is discontinued only when the INR is ≥2 for 2 consecutive days (Linkins et al. 2012; Warkentin, Greinacher et al. 2008; Warkentin et al. 1997; Srinivasan et al. 2004). If warfarin therapy has been initiated before HIT is diagnosed, reversal with an oral or an intravenous dose of vitamin K is advised to prevent VLG or WISN (Linkins et al. 2012; Warkentin, Greinacher et al. 2008).

All DTIs prolong the INR, largely a result of the differences in the molar concentrations required to achieve the desired inhibition of thrombin (Warkentin, Greinacher et al. 2005). An increased INR is most pronounced with argatroban (the manufacturer recommends an INR ≥4.0 during cotherapy) and least affected by lepirudin. As a result, the INR (and aPTT) should be checked once the targeted INR is reached (assuming a minimum 5-day overlap with a DTI) and only after holding the DTI 4–6 h. If the INR is within the targeted range (generally 2–3 for lepirudin or ≥4 with argatroban) and the aPTT is at baseline, the DTI can be safely discontinued (Warkentin, Greinacher et al. 2008).

The appropriate length of therapy with warfarin following an episode of HIT is not well defined, but most authors recommend at least 3–6 months.

Reducing the Incidence of HIT

The use of LMWH or fondaparinux as alternatives to UFH for VTE prophylaxis and/or treatment should help reduce the overall incidence of HIT because these anticoagulants are less likely to trigger this response. In addition, early transition to warfarin (minimizing the amount of time the patient receives UFH or LMWH) for acute DVT or PE is also advised. Patients receiving UFH should have their platelet counts monitored regularly as advised by the 9th ACCP guidelines. These guidelines recommend that individuals who are considered at high risk of developing HIT (incidence >1 %) should have a platelet count examined at baseline and every 2 or 3 days from days 4 to 14 (or until UFH is stopped). These include individuals receiving UFH for prophylaxis or therapeutic dosage as well as

cardiac surgery patients and patients with cancer (Linkins et al. 2012). In addition, if there has been a recent exposure (within the last 100 days), these same guidelines advise checking a platelet count at baseline and within 24 h of administration (Linkins et al. 2012). Unlike the 9th ACCP guidelines which do not recommend routine monitoring for patients receiving LMWH, the 8th ACCP guidelines and the British Committee for Standards in Haematology advise monitoring platelet counts for postoperative patients receiving LMWH (Linkins et al. 2012; Warkentin, Greinacher et al. 2005; Keeling et al. 2006).

Special Conditions

Isolated HIT

Isolated HIT was previously treated simply by discontinuing UFH or LMWH; however, this approach has been clearly shown to leave patients at increased risk for new thromboembolic events with rates of 17–55 % (Linkins et al. 2012; Warkentin, Greinacher et al. 2008; Lewis et al. 2003; Wallis et al. 1999; Warkentin and Kelton 1996). Current 2012 ACCP guidelines advise treatment with a DTI (lepirudin or argatroban) for isolated HIT followed by a short course of warfarin. These guidelines do not comment on the need for a venous duplex ultrasound of the lower extremities (because of the high frequency of asymptomatic DVT associated with HIT) as was advised in the 2008 guideline (Linkins et al. 2012; Warkentin, Greinacher et al. 2008).

Clinical Vignette 3

A 75-year-old male undergoes three-vessel coronary artery bypass surgery. He has a decline in his platelet count immediately postoperatively (from a baseline of 275,000/μl to 145,000/μl) followed by a short recovery until day 6 when his platelet count drops again. On day 9 post surgery, his platelet count is 43,000/μl and you are consulted (see Fig. 14.4).

HIT and Cardiac Surgery

Patients undergoing cardiac surgery pose several problems for the clinician managing the individual with acute HIT including the choice of anticoagulation for the person who requires urgent CABG and correctly diagnosing HIT in the postoperative setting where thrombocytopenia may be a result of several other conditions.

The incidence of HIT is approximately 1–3 % following CABG; however, antibody formation is much higher, approaching 50 % or greater immediately following surgery. This can pose a potential for overdiagnosing HIT if one relies entirely on laboratory findings (Arepally and Ortel 2006). In this setting, a clue to the correct diagnosis is that HIT classically presents as a biphasic pattern of platelet recovery: an initial postoperative decline followed by recovery only to lead to a new drop in the platelet count generally on day 5 or more post surgery (Arepally and Ortel 2006; Lillo-Le-Louet 2004). Other clues may include unusual or unexpected thromboembolic events, prolonged thrombocytopenia, immunoassay values >1.0 OD, or a positive functional test.

Anticoagulation for the HIT patient requiring urgent CABG also presents a difficult dilemma as current available alternative anticoagulants are limited by their lack of an antidote and the need for special intraoperative monitoring. Several trials using bivalirudin in both "on-pump" and "off-pump" cardiac surgery have been completed, and the 9th ACCP guidelines advocate its use over other available DTIs in patients with acute or recent HIT (Dyke et al. 2007; Koster et al. 2007).

Re-exposure to UFH in patients with HIT (or a history of HIT) is an area of controversy but has been used successfully for CABG when the surgeon feels that there is no good alternative anticoagulant available. The most recent ACCP guidelines advise avoiding surgery until the heparin antibodies disappear (Linkins et al. 2012). Once testing confirms their absence, re-administration of UFH (during the procedure) appears safe (Warkentin and Kelton 2001; Potzsch et al. 2000).

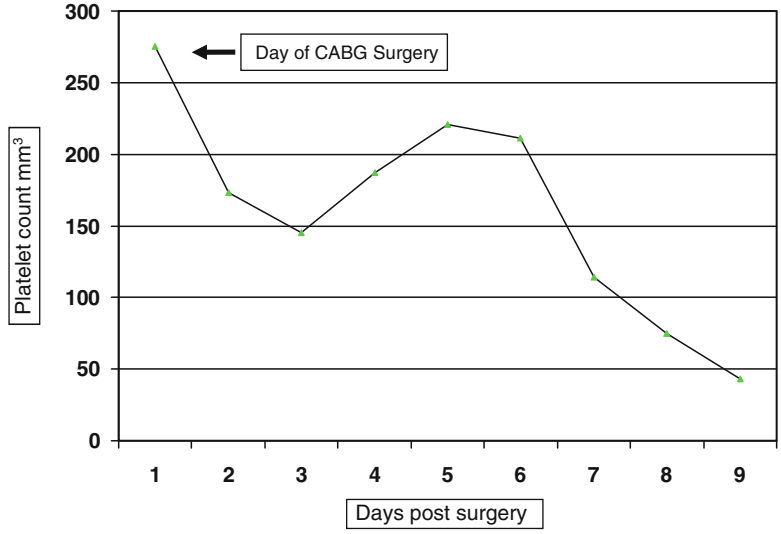

Fig. 14.4 Platelet count post surgery

Conclusion

HIT is a serious adverse reaction to UFH or LMWH that if not promptly recognized and treated can lead to devastating complications. Thrombocytopenia is the most common presentation, whereas thrombosis develops in over one-half of all patients. Lower extremity DVT and PE are the most frequent venous thrombotic events, while acute limb occlusion occurs more often than stroke or myocardial infarction.

HIT is a clinicopathologic syndrome. A high clinical suspicion, a pretest probability score using the 4Ts, and positive laboratory testing will help confirm the diagnosis. Immediate discontinuation of UFH or LMWH and the initiation of a DTI followed by warfarin therapy once the platelet count has recovered are recommended.

There are several approaches to prevent HIT. Close monitoring of the platelet count while the patient is receiving UFH should be performed, and although monitoring the platelets of patients receiving LMWH is not currently recommended in the United States, it is advised by the British. The use of alternatives to UFH (LMWH or fondaparinux) as prevention or treatment should also be considered given their lower incidence of HIT.

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Surgery on Patients on Antiplatelet Agents

15

Michael A. Militello

Clinical Vignette

A 65-year-old male has recently been evaluated for severe hip pain that has progressively become worse over the past couple of years and was determined that he will need to have the hip replaced. This patient has multiple medical problems including hypertension, hyperlipidemia, and diabetes mellitus. Two months ago he had a non-ST segment elevation myocardial infarction and two drug eluting stents (DES) were placed to the proximal right coronary artery. He was placed on aspirin 81 mg daily and clopidogrel 75 mg daily. The orthopedic surgeon would like to proceed with the hip replacement but is concerned that if he does not stop the dual antiplatelet therapy (DAPT) the bleeding risk will be too high and if he does stop the therapy then the patient is at risk of stent thrombosis. What considerations need to be considered when determining the next course of action?

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Introduction

Antiplatelet drugs are commonly used in the management of thrombotic diseases including transient ischemic attacks (TIA), stroke, acute myocardial infarction (AMI), acute coronary syndrome (ACS), peripheral arterial disease (PAD), percutaneous coronary intervention (PCI), cardiac and vascular surgery, primary and secondary cardiovascular disease prevention, and atrial fibrillation.

Heart disease is the leading cause of death and morbidity in the USA. In 2009 about 1 of every 6 deaths were attributed to coronary artery disease accounting for nearly 400,000 deaths (Go et al. 2013). In 2010 it was estimated that 492,000 patients had percutaneous coronary intervention (PCI) with stents placed in most of the cases (Go et al. 2013). With the advent of stents and the need for dual antiplatelet therapy (DAPT) to prevent stent thrombosis (ST), PCI has become the primary mode of coronary artery revascularization. The obvious advantages of this mode of revascularization are not without its limitations and difficulties. One of the major limitations to PCI with stent placement is the need for long-term DAPT. Early cessation of DAPT can lead to ST, which can cause myocardial infarction and potentially death (Grines et al. 2007). Continuation of these agents in patients undergoing certain invasive procedures can increase the risk of bleeding. The most common reasons for early discontinuation of DAPT include patient

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noncompliance and the need for noncardiac surgery (van Werkum et al. 2009; Ferreira-Gonzalez et al. 2012; Spertus et al. 2006). It is estimated that between 4 and 8 % of patients undergoing coronary stent placement will undergo some type of noncardiac surgery (NCS) within the first year of stent deployment (Anwaruddin et al. 2009; Cruden et al. 2010; Berger et al. 2010). Thus, the risk of early and late ST can depend on the timing of surgery versus stent deployment. Surgery can produce stress responses including stimulation of the sympathetic nervous system, release of endogenous factors that can cause sheer stress, activate platelets, and cause vascular reactivity potentially increasing the risk of cardiovascular events (Desborough 2000). Also, there is a propensity towards hypercoagulability with increased clotting factors and decreased endogenous fibrinolysis (Bradbury et al. 1997; Mahla et al. 2001; Diamantis et al. 2007; Davenport 2007). All of the above factors may lead to increased risk of ST when DAPT is discontinued. Timing of DAPT discontinuation after stent deployment should be considered to prevent ST or the risks of major bleeding if DAPT were not to be interrupted. A recent review suggests that patients undergoing NCS after bare metal stent (BMS) or DES placement have the highest risk of major adverse clinical events within the first 6 weeks after BMS placement and up to 6 months after DES. This risk decreases over time to rates of 2.8 % in patients where NCS was done between 90 and 360 days after PCI (Singla et al. 2012; Kleinman 2012).

The American Heart Association/American College of Cardiology (AHA/ACC) recommendations for ST-segment elevation myocardial infarction undergoing primary PCI recommend a loading dose of aspirin to be continued indefinitely and a loading dose of either clopidogrel or prasugrel or ticagrelor to be continued at their respective maintenance doses for 1 year regardless of the type of stent placed (O'Gara et al. 2013). Similar recommendations are discussed in the non-ST segment elevation myocardial infarction guidelines, PCI guidelines and the American College of Chest Physicians guidelines for antithrombotic therapies (Wright et al. 2011).

However, there are situations in which DAPT may need to discontinued early and the following are issues to be considered: the urgency of the surgery or procedure, the type of stent placed (BMS versus DES), and as mentioned previously, the time from stent placement to the timing of DAPT discontinuation.

Antiplatelet Medications

Aspirin is a nonselective cyclooxygenase (COX) inhibitor that irreversibly binds to the COX enzyme in the platelet inhibiting the conversion of arachidonic acid to thromboxane A_2 (see Table 15.1). Thromboxane A2 is a vasoconstrictor and causes platelet aggregation. The duration of inhibition of aspirin is the life of the platelet, generally considered to be around 6–8 days. Aspirin is absorbed after oral administration with peak levels occurring 30–40 min after ingestion. The

Table 15.1 General information for selected antiplatelet antagonist

Drug	Class	*T½*	Duration of antiplatelet effect	Reversible inhibition
Aspirin	COX-inhibitor	~3 h	Life of platelet	No
Clopidogrel	$P2Y_{12}$ inhibitor	6 h (clopidogrel) 0.6–0.7 h (active metabolite)	Life of platelet	No
Prasugrel	$P2Y_{12}$ inhibitor	7–8 h (active metabolite)	Life of platelet	No
Cangrelor	$P2Y_{12}$ inhibitor	2.5–5.5 min	Reverses quickly	Yes
Ticagrelor	$P2Y_{12}$ inhibitor	7 h (ticagrelor) 9 h (active metabolite)	Reverses within days	Yes
Eptifibatide	Glycoprotein IIb/IIIa	2.5 h	4–9 h	Yes
Tirofiban	Glycoprotein IIb/IIIa	2 h	4–9 h	Yes

major adverse effect of aspirin is bleeding, most commonly from the gastrointestinal tract. This risk can be decreased by the use proton pump inhibitors (PPI's) (Ng et al. 2010).

Commonly used antiplatelet medications work synergistically with aspirin by inhibiting the adenosine diphosphate (ADP) receptor found on the surface of the platelet. The purinergic receptor $P2Y_{12}$ or ADP receptors when stimulated lead to the expression of the glycoprotein IIb/IIIa receptors which are necessary for binding of fibrinogen to cross link platelets during the formation of a stable thrombus. There are two main classes of $P2Y_{12}$ receptors inhibitors the thienopyridines (ticlopidine, clopidogrel, and prasugrel) and the non-thienopyridine (ticagrelor and cangrelor). Both classes bind to the $P2Y_{12}$ receptors to provide potent platelet inhibition but bind at different sites on this receptor.

Thienopyridines

Clopidogrel and prasugrel produce irreversible inhibition of platelet aggregation through binding to the adenosine diphosphate receptors and take approximately 3–7 days after daily administration to reach maximal effects; however, this process is shortened by an initial loading dose. The usual daily dose of clopidogrel is 75 mg given daily. A loading dose of 300–600 mg is commonly used prior to interventional procedures to achieve a therapeutic effect sooner. Both agents are pro-drugs that need to be converted to their active metabolites. Clopidogrel needs to undergo a two step process to become biologically active, whereas prasugrel only needs one metabolic transformation (Abraham et al. 2010). It is eliminated by the feces and urine. Clopidogrel has several adverse effects, mainly bleeding. This is especially of concern around the time of cardiac surgery when an increased need for surgical reexploration and the use of blood products has been reported. Other side effects include diarrhea, rash and rarely neutropenia and thrombotic thrombocytopenia purpura (TTP).

Ticlopidine, a first-generation thienopyridine, is seldom used today because of its neutropenic and TTP potential side effects and slower onset of action compared to clopidogrel and prasugrel.

Prasugrel, like clopidogrel, is an irreversible inhibitor of the $P2Y_{12}$ ADP receptor. It too is a prodrug that is rapidly absorbed and metabolized. Prasugrel's onset occurs more quickly and to a greater extent when compared with clopidogrel (Payne et al. 2007). Bleeding is the most significant side effect of prasugrel and it is recommended that prasugrel be discontinued 5–7 days prior to high bleeding risk surgical procedures (Antman et al. 2008; Roe et al. 2012).

Reversible $P2Y_{12}$ Receptor Antagonists

Ticagrelor is an orally administered, reversible inhibitor of the $P2Y_{12}$ receptor. Absorption is rapid and does not require biotransformation to an active compound unlike clopidogrel and prasugrel. Ticagrelor is metabolized into an equally potent active metabolite (AR-C124910XX) by the cytochrome P450 3A4 enzymes, however only achieves levels of one-third that of the parent compound (van Giezen and Humphries 2005; Storey et al. 2007). The half-lives of ticagrelor and AR-C124910XX are similar ranging from 7 to 12 h. A single loading dose of ticagrelor produces peak inhibition of platelet aggregation within 2 h. The offset of ticagrelor is between 72 and 120 h depending on the type of platelet inhibition assay employed, suggesting that this time period is required to decrease the risk of bleeding complications in patients undergoing noncardiac surgery. In the PLATO trial (Wallentin et al. 2009), patients who were to undergo cardiothoracic surgery had ticagrelor discontinued for at least 5 days prior to surgery.

Cangrelor is a reversible inhibitor of platelet function. It is given intravenously. Cangrelor reaches a steady state within 30 min without a loading dose. It has an elimination half-time of less than 9 min. Platelet function returns to normal within 60 min (Angiolillo et al. 2012). It is not currently available in the USA.

Glycoprotein IIb/IIIa Inhibitors

Abciximab is a monoclonal chimeric antibody that essentially irreversibly binds to the glycoprotein

IIb/IIIa receptor. It has a short half-life, however a long duration of effect secondary to its high binding affinity to the receptor. Platelet function returns to normal within 48–72 h of discontinuing therapy without platelet transfusion. Platelet transfusion can reverse the effects of abciximab due to its irreversible nature and no active metabolites. Eptifibatide and tirofiban reversibly bind to the glycoprotein IIb/IIIa receptors. Their half-lives are relatively short (2–2.5 h) in patients with normal renal function and prolongs in patients with renal insufficiency. These agents are dosed based on weight, and are continuous infusions. Once the drugs are discontinued, platelet function returns to normal within 6–12 h in patients with normal renal function, however may be prolonged in patients with severe renal insufficiency. The most common side effect of these agents is bleeding. The most common bleeding complaints include epistaxis, genitourinary and gingival bleeding. Other more serious but rare bleeding complication includes alveolar/pulmonary hemorrhages. Infusion durations of these agents have been limited to 96 h; however, there are occasions where longer infusions have been use.

Strategies for Patients Requiring Discontinuation of DAPT for Noncardiac Surgery

The most practical approach to patients requiring noncardiac surgery and disruption of DAPT would be to delay, if possible, the surgery until after 1 month for BMS and 12 months for DES. Patients should have their $P2Y_{12}$ therapy discontinued for 5–7 days before surgery and maintain aspirin if possible. Of the $P2Y_{12}$ inhibitors, prasugrel should be discontinued 7 days prior to surgery; however, clopidogrel and ticagrelor may be discontinued for 5 days as their effects dissipate more quickly. Ticagrelor's effects on platelets reverse more quickly in less than 5 days. However in the PLATO trial, which compared ticagrelor to clopidogrel in patients with ACS, the study design recommended to discontinue therapy 5 days before cardiovascular surgery (Wallentin et al. 2009).

If surgery cannot be delayed then the type of stent needs to be taken into consideration prior to discontinuation of DAPT. Patients who have received a BMS may have DAPT interrupted while continuing aspirin after 4–6 weeks and reinitiated once hemostasis is achieved (Grines et al. 2007). In contrast, patients who had a DES placed should wait, if possible, for 12 months (Grines et al. 2007).

Finally, if surgery cannot be delayed until the recommended duration of DAPT therapy is complete, the risks of bleeding versus the risk of ST needs to be considered. There are little data available to make general recommendations for urgent/emergent scenarios. Some institutions utilize glycoprotein IIb/IIIa inhibitors as a bridge prior to the surgery. Heparin and low-molecular-weight heparin have also been used; however, formal recommendations are lacking. Initiating either tirofiban or eptifibatide after DAPT has been discontinued and stopping the GP IIb/IIIa inhibitor 4–6 h prior to surgery has also been recommended. Currently, there have been no prospective randomized trials evaluating the use of GP IIb/IIIa inhibitors for bridging therapy. An initial case series of three patients with DES bridged with tirofiban demonstrated no cases of ST or major bleeding events (Broad et al. 2007). Ben Morrison (Ben Morrison et al. 2012) reviewed the use of GP IIb/IIIa inhibitors as a bridge for patients with DES undergoing either cardiac or noncardiac surgery. The primary endpoint of this study was the development of ST. Other endpoints included major and minor bleeding, the occurrence of ACS and death. Of the 19 patients included in this study, 6 patients underwent noncardiac surgery and 13 patients had cardiac surgery. No patients in the noncardiac surgery arm had an occurrence of ST or major bleeding; however in the cardiac surgery patients, there were seven episodes of major bleeding and one episode of minor bleeding without any occurrences of ST. Rassi et al. (2012), reviewed 100 consecutive patients receiving eptifibatide as a bridge to both noncardiac and cardiac surgery matched with historical controls. This study was primarily a safety study with the main endpoint being the number of units of blood received dur-

ing the hospital admission. Other endpoints included transfusion of platelets or fresh frozen plasma, major adverse cardiovascular events, intracranial and intraocular hemorrhage, gastrointestinal bleeding, severe epistaxis, surgical wound bleeding, and the need for return to the operating room. In this study, there were no significant differences between the groups in terms of the primary endpoint and secondary endpoints. This trial may not have had a sample size large enough to determine a difference. There were no events of ST in the groups. These studies suggest that bridging with a glycoprotein IIb/IIIa inhibitor does not increase the risk of major bleeding and may protect against ST in patients needing discontinuation from DAPT within the first year of stent implantation. However, there are a number of limitations to these trials including the retrospective nature of the trials, with limited comparisons to other therapies. There is one small prospective phase II trial evaluating tirofiban as a bridge in patients who had received a DES within the previous 1–12 months that required discontinuation of DAPT. Savonitto (Savonitto et al. 2010) systematically evaluated patients requiring discontinuation of oral therapy. They discontinued clopidogrel 5 days prior to surgery and initiated tirofiban on preoperative day minus 4. They also evaluated perioperative bridging with initiation of either clopidogrel or tirofiban postoperatively. The primary endpoint was the composite of cardiovascular death, MI, ST, or the need for surgical reexploration because of bleeding. No patients met the primary endpoint in this trial and there was one patient who developed major bleeding. However, 20 % ($n=6$) of the study subjects required either preoperative or postoperative blood transfusions.

The two greatest considerations in discontinuing a patient's DAPT are ST and risk of perioperative and postoperative bleeding. The risks of each need are to be considered in making the decision to interrupt DAPT. Procedures that are considered low risk of bleeding, such as endoscopy, dental extraction and surgery, minor orthopedic surgery, and cutaneous surgery may not necessitate the discontinuation of DAPT. However, surgery and procedures with intermediate and high risk of bleeding will need consideration of discontinuing DAPT and maintaining aspirin if possible through the perioperative course.

Case Conclusion

In this situation it would be best to post pone surgery until one year after the sent had been placed. If surgery was required sooner then waiting as long as possible and consulting with the provider that placed the stent would be most appropriate.

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Newer Oral Anticoagulants

16

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Clinical Vignettes

Clinical Vignette 1: A 28-year-old woman is 6 weeks postpartum and breastfeeding. She develops left leg swelling and is diagnosed with deep vein thrombosis (DVT). She asks if she can take one of the new oral anticoagulants that she has heard about instead of taking warfarin.

Clinical Vignette 2: Your patient with atrial fibrillation has been on warfarin for many years and has consistently had difficulty maintaining a therapeutic international normalized ratio (INR). He admits to missing doses occasionally despite being advised of the importance of being compliant with dosing instructions. During his office visit he asks about switching to one of the new medications which he has heard about on television.

Clinical Vignette 3: Your patient with mild renal impairment (creatinine clearance of $>50 \leq 80$ ml/min) on dabigatran for atrial fibrillation is going to have surgery and therefore has held his medication for 48 h. Upon admission to the hospital the surgeon is concerned about surgical and postoperative bleeding risks. What laboratory test can be run prior to surgery to ensure that no residual anticoagulant effect remains?

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Dabigatran Etexilate

Dabigatran etexilate is administered orally as a prodrug and converted to the active compound, dabigatran. It is a competitive reversible direct inhibitor of both free and clot bound thrombin. It has a US FDA approval for prevention of stroke and systemic embolization in patients with nonvalvular atrial fibrillation. It has an additional indication in Canada and the UK for VTE prevention after elective hip and knee surgery. A predictable pharmacokinetic profile allows for fixed dosing with no need for coagulation monitoring or dose adjustments.

Key Clinical Trials

Dabigatran was compared directly to adjusted dose warfarin in patients with atrial fibrillation. In a noninferiority trial enrolling 18,113 patients with atrial fibrillation and CHADS2 score (Congestive heart failure, Hypertension, Age ≥75 years, Diabetes mellitus, Stroke, or otherwise history of arterial thromboembolism) of two or more, patients were randomized to receive dabigatran (either 110 or 150 mg twice daily) or adjusted dose warfarin with a median follow-up of 2 years and primary outcome of stroke or systemic embolism (Connolly et al. 2009). The rate of stroke and systemic embolism was similar between warfarin and the 110 mg dabigatran dose with a lower rate of major hemorrhage in

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patients receiving 110 mg of dabigatran. There was a lower rate of stroke and systemic embolism in patients receiving the 150 mg dose of dabigatran (superior) compared to warfarin with similar rates of major hemorrhage. At either dose of dabigatran, the rate of intracranial hemorrhage was 1/3 of the rate in patients taking warfarin.

Dabigatran has been studied in three large phase 3 trials for prevention of VTE following hip or knee surgery altogether randomizing more than 8,000 patients to dabigatran versus enoxaparin (RE-MODEL and RE-MOBILIZE after knee surgery, RE-NOVATE after hip surgery). Patients were randomized to receive either dabigatran (150 or 220 mg once daily) vs. enoxaparin (40 mg once daily or 30 mg twice daily). The primary endpoint in all trials was total VTE events, and a composite of venographic DVT, symptomatic DVT, and all-cause mortality. The duration of thromboprophylaxis was 6–10 days in RE-MODEL, 12–15 days in RE-MOBILIZE, and 28–35 days in RE-NOVATE. In a meta-analysis of all three trials the higher dose dabigatran (220 mg once daily) was noninferior to enoxaparin (40 mg once daily) with a similar rate of major bleeding. Interestingly both doses of dabigatran did not achieve noninferiority compared to enoxaparin (30 mg twice daily) in the RE-MOBILIZE trial. This data led to approval of dabigatran 220 mg once daily for thromboprophylaxis following hip or knee surgery in Europe and Canada (Eriksson et al. 2007a, b; Ginsberg et al. 2009; Wolowacz et al. 2009).

Dabigatran (150 mg twice daily) has been studied for the treatment of acute VTE; acute proximal DVT or pulmonary embolism (PE) or both in phase 3 clinical trials (RE-COVER, RE-COVER II) compared with adjusted dose warfarin (Schulman et al. 2009). Data from the first RE-COVER study group is published. The study was a double-blind, noninferiority design randomizing 2,539 patients to either treatment arm for a 6 month course of therapy. An initial 5–10 day course of parental anticoagulation (either unfractionated heparin or low-molecular-weight heparin) was administered to all patients. The primary endpoints were composite recurrent VTE and VTE-related mortality. Dabigatran was found to be noninferior to warfarin with a similar safety profile in terms of major bleeding risk.

To date, data from RE-COVER 2 has only been presented in abstract form. This study again compared fixed dose dabigatran directly with adjusted dose warfarin for the treatment of acute VTE for 6 months and confirmed noninferiority of dabigatran compared to adjusted dose warfarin with a lower risk of clinically relevant bleeding (Schulman 2012). Additional phase 3 studies have been conducted evaluating the efficacy and safety of extending anticoagulant therapy with dabigatran compared to warfarin (RE-MEDY) or placebo (RE-SONATE). Both studies were double blind and randomized, comparing dabigatran 150 mg twice daily to either warfarin (active-control study) or placebo (placebo-control study). All patients had completed at least a 3 month course of anticoagulation for symptomatic proximal VTE. The active-control study randomized 2,856 patients and demonstrated a rate of recurrent VTE which was considered noninferior for dabigatran (1.8 % vs. 1.3 %) with significantly less frequent major or clinically relevant bleeding in patients treated with dabigatran (Schulman et al. 2013). The placebo-control study randomized 1,343 patients demonstrating a significant reduction in recurrent VTE in patients receiving dabigatran but with a significantly greater rate of major or clinically relevant bleeding. The authors conclude that dabigatran is effective for the extended treatment of VTE with a lower risk of major or clinically relevant bleeding compared to warfarin but a higher risk of bleeding compared to placebo. Interestingly, there was a higher rate of acute coronary syndrome in the dabigatran arm of the active-control study (0.9 % vs. 0.2 %, $P = 0.02$). The authors point out that this phenomenon was not seen in the placebo-control study. A similar finding was suggested in the RE-LY study (dabigatran vs. warfarin in patients with atrial fibrillation), but further evaluation of the data seemed to eliminate its significance. However, a meta-analysis of seven trials showed significantly higher risk of myocardial infarction or ACS associated with dabigatran (Uchino and Hernandez 2012).

Pharmacokinetics

Dabigatran etexilate is a small synthetic molecule administered orally as a prodrug which is converted in the liver to the active compound, dabigatran. It is a competitive reversible direct inhibitor of both free and clot bound thrombin through binding of the active site of the thrombin molecule. It has a rapid onset of action (peak plasma concentration and anticoagulant effect) which is achieved within 1.5–3 h of dosing. Its elimination half-life is 14–17 h (longer in the elderly). Dabigatran is excreted unchanged by the kidneys (80 %) and eliminated via bile (20 %). Dabigatran does not induce or inhibit cytochrome P450 drug metabolizing enzymes. It is a substrate for P-gp (permeability glycoprotein) which gives rise to clinically important drug interactions (i.e., ketoconazole and amiodarone increase plasma concentrations). Concomitant administration of strong P-gp inhibitors (i.e., itraconazole and protease inhibitors like ritonavir) with dabigatran requires great caution or is contraindicated. Moderate inhibitors of P-gp (i.e., macrolides like clarithromycin) are not considered to have a clinically important interaction. Rifampicin is a P-gp inducer causing a decreased anticoagulant effect and because dabigatran requires a low pH for absorption, proton pump inhibitors (PPI) may also decrease its anticoagulant activity. Dabigatran has low plasma protein binding (35 %) thus is not affected by displacement interactions which can occur when a second medication is administered. Food prolongs time to peak plasma levels by about 2 h (Poulsen et al. 2012).

Coagulation Assays

Because of the predictable pharmacokinetics exhibited by dabigatran routine monitoring is not necessary. However, there may be circumstances that arise in which knowledge about the anticoagulation status of a patient is needed. Situations that may arise include active bleeding, overdose and when urgent or emergent surgery is needed. Dabigatran inhibits thrombin mediated conversion of fibrinogen to fibrin therefore will affect all routine coagulation assays. The activated partial thromboplastin time (aPTT) assesses the intrinsic pathway of the coagulation cascade and prolongs as dabigatran plasma concentration increases, but this response flattens at high contraindications. The aPTT is relatively insensitive within the range of dabigatran plasma concentrations seen in patients. Therefore, it may be useful qualitatively, confirming presence of dabigatran anticoagulant activity, but it is not appropriate for quantifying anticoagulant effects, especially at high plasma concentrations. The prothrombin time (PT) assesses the clotting time in the extrinsic pathway of the coagulation cascade and is insensitive to prophylactic doses of dabigatran and elevates slightly at therapeutic doses. Therefore, it is of limited value in determining the anticoagulant status of a patient. The thrombin time (TT) is a direct measure of thrombin activity in plasma and has a linear dose response over therapeutic concentrations. However, standardized reference ranges are not possible because reagents differ among laboratories; furthermore, as dabigatran concentrations increase, the test will often exceed the maximum measurement time of coagulometers, and thus thrombin time may not be ideal (too sensitive) in the emergent setting (Stangier et al. 2007). It is most useful to determine presence of dabigatran activity rather than to quantify the level. The activated clotting time (ACT) is a quantitative assay using whole blood samples. There is a linear concentration-dependent increase in ACT up to 250 ng/ml and then the response flattens similar to that seen with aPTT testing, limiting its utility. The Hemoclot® thrombin inhibitor assay (Hyphen Biomed, France®) is a diluted TT assay allowing for quantitative measurements of DTI activity in plasma based on a constant defined concentration of thrombin. Diluted test plasma is mixed with normal pooled human plasma and clotting is stimulated by adding highly purified human thrombin. There is a direct linear relationship between dabigatran concentration and clotting time (about 30–75 s). The test can be used for any DTI (Stangier and Feuring 2012).

The ecarin clotting time (ECT) measures thrombin generation. Ecarin is isolated from snake venom (*Echis carinatus*) which activates prothrombin resulting in generation of meizothrombin (precursor of thrombin). The DTIs inhibit the thrombin-like activity of meizothrombin therefore the ECT can directly measure DTI activity. There is a linear relationship between ECT prolongation and DTI plasma concentration. The ECT has been demonstrated to be a sensitive test for measuring DTI anticoagulant activity however it is mostly used as a research tool and is not yet widely available or validated for routine clinical use in emergent settings (Van Ryn et al. 2010).

Reversal of Dabigatran

Under most circumstances, withholding drug and waiting for its activity to dissipate is adequate without the need for a specific antidote. The length of time to withhold dabigatran will depend on the patient's renal function and reason for anticoagulation activity reversal. The half-life of dabigatran in patients with normal to mildly impaired renal function (creatinine clearance above 50 ml/min) is 15 h (range 12–34 h); therefore, for standard risk elective surgery, one should hold dabigatran for 24 h prior to surgery. If the surgery or patient is considered higher risk for bleeding and complete hemostasis is desired then the drug should be held 2–4 days before surgery (Van Ryn et al. 2010). The half-life of dabigatran in patients with moderately impaired renal function (creatinine clearance 30–50 ml/min) is 18 h (range 13–23 h) and holding the drug for 2 days is suggested for standard bleeding risk procedures, and at least 4 days for higher bleeding risk patients. For patients at greater risk of bleeding, measurement of a TT 6–12 h before surgery can be performed and if elevated indicates persistence of DTI activity and the elective surgery should be postponed. Patients with severe renal dysfunction (creatinine clearance <15 ml/min) should not be prescribed dabigatran. In case of recent overdose (within 1–2 h) activated charcoal may be given. If the overdose occurred more than 2 h from the presentation, further removal of dabigatran from plasma by hemoperfusion over a charcoal filter is under investigation. If a patient on dabigatran presents with bleeding, as with any anticoagulant, the severity of bleeding, timing of last dose, degree of coagulopathy, and clinical response to supportive measures should guide medical intervention decisions. The medication should be withheld, the site of bleeding should be compressed, and surgical hemostasis should be provided if possible. Transfusion of blood products should be provided as indicated (red blood cells, fresh frozen plasma). Dabigatran plasma concentrations decline relatively quickly following discontinuation of the drug. If supportive measures are inadequate, hemodialysis may be an option, although there is limited guiding data. In cases of the need for more rapid reversal of anticoagulant activity, especially in a patient with renal impairment dialysis has been shown to remove 62 % of drug at 2 h and 68 % at 4 h (Stangier et al. 2010). There is limited and inconsistent data on the utility of recombinant coagulation factor VIIa in the reversal of anticoagulant activity from direct thrombin inhibitors (Marlu et al. 2012). Additionally, use of prothrombin complex concentrates (PCC) is under investigation. In one randomized, double-blind, placebo controlled study, dabigatran's anticoagulant effect was not reversed with PCC (Eerenberg et al. 2011).

Rivaroxaban

Rivaroxaban is a new oral anticoagulant which works chiefly by direct, selective, and reversible inhibition of factor Xa, thereby inhibiting platelet activation and fibrin clot formation. In 2011 the US FDA approved rivaroxaban for prevention of stroke and systemic embolization in patients with non-valvular atrial fibrillation and for prevention of VTE in patients undergoing knee or hip replacement surgery. In 2012 the US FDA expanded approval of rivaroxaban to include treatment of VTE

Key Clinical Trials

In a large double-blind clinical trial of 14,264 patients with nonvalvular atrial fibrillation at elevated risk for stroke with a CHADS2 score of 2 or above were randomized to either rivaroxaban (20 mg once daily) or dose-adjusted warfarin (Patel et al. 2011). Rivaroxaban was found to be noninferior to warfarin at preventing stroke or systemic embolization with no difference in the risk of major bleeding. In fact, there was significantly less intracranial hemorrhage (0.5 % vs. 0.7 %, $P=0.02$) and fatal bleeding (0.2 % vs. 0.5 %, $P=0.003$) in the rivaroxaban group (ROCKET-AF clinical trial).

In a pooled analysis of four phase 3 double-blind, randomized studies (RECORD1–4) directly comparing rivaroxaban (10 mg once daily) to enoxaparin (40 mg once daily or 30 mg twice daily) for thromboprophylaxis following hip or knee arthroplasty, including 12,729 patients, rivaroxaban had superior efficacy at reducing the composite end point of symptomatic VTE and all-cause mortality after hip or knee arthroplasty. There was no significant difference in the incidence of major bleeding between rivaroxaban and enoxaparin (Turpie et al. 2009, 2011; Eriksson et al. 2008; Kakkar et al. 2008; Lassen et al. 2008). In another phase III trial, rivaroxaban (10 mg once daily) was compared to enoxaparin (40 mg once daily) in acutely ill medical patients (Cohen et al. 2013). The patients were randomized if they were 40 years of age or older and hospitalized with an acute medical illness. Thromboprophylaxis was provided for 10 ± 4 days with either drug and an extended treatment with rivaroxaban out to 35 ± 4 days was compared to placebo. The primary efficacy outcome was VTE occurring up to 10 days for the noninferiority test and up to 35 days for the superiority test. A total of 8,101 patients underwent randomization with just under 3,000 patients being randomized to each of the two medication groups. At 10 days there was a difference in the incidence of the primary efficacy outcome. Extended therapy rivaroxaban resulted in statistically fewer VTE events compared to enoxaparin followed by placebo at 35 days. Rivaroxaban was found to be noninferior compared to enoxaparin for thromboprophylaxis in acutely ill medical patients; however, there was significantly more major bleeding associated with rivaroxaban at both 10 and 35 days.

Three large phase 3 trials have been conducted to assess for treatment of VTE (EINSTEIN-EXT), treatment of acute DVT (EINSTEIN-DVT) and acute PE (EINSTEIN-PE) with rivaroxaban. Rivaroxaban (15 mg twice daily for 3 weeks followed by 20 mg once daily) was compared to enoxaparin followed by adjusted dose warfarin for treatment of acute deep DVT in an open-label, randomized, noninferiority study. Treatment duration was 3, 6, or 12 months in patients with acute symptomatic DVT. The study included 3,449 patients, 1,731 were randomized to rivaroxaban and 1,718 were treated with warfarin. Rivaroxaban was noninferior at preventing recurrent VTE with identical rates of major bleeding or clinically relevant nonmajor bleeding. The extended treatment arm of the study was double-blind and randomized and designed to assess for superiority of continued rivaroxaban (20 mg once daily) compared to placebo for an additional 6–12 months. There were 602 patients in the rivaroxaban group and 594 in the placebo group. Superior efficacy was demonstrated with eight recurrent VTE events occurring in the treatment group compared to 42 events in the placebo group. There was no statistical difference in bleeding rates (Bauersachs et al. 2010). In a similarly designed noninferiority study, 4,832 patients with acute symptomatic PE with or without DVT were randomized to either rivaroxaban or standard therapy (enoxaparin followed by adjusted dose vitamin K antagonist) for 3, 6, or 12 months. Rivaroxaban again proved noninferior with a statistically significant lower rate of major bleeding (Buller et al. 2012).

Rivaroxaban has also been evaluated for ACS. In the ATLAS ACE-TIMI 51 study (Mega et al. 2012) 15,526 patients with non-ST elevation myocardial infarction (MI), and ST MI or unstable angina were randomized to either rivaroxaban (2.5 mg twice daily or 5 mg twice daily) or placebo along with standard therapy.

Patients were enrolled within 7 days of an event, after intervention and stabilization. The primary endpoint was a composite of cardiovascular death, MI, or stroke. Rivaroxaban was effective at reducing the primary composite endpoint at both doses. The lower dose reduced cardiovascular death and all-cause mortality but not the higher dose and there were increased rates of major bleeding and intracranial hemorrhage but not fatal bleeding (Mega et al. 2012).

Pharmacokinetics

Rivaroxaban is a reversible, direct inhibitor of not only free factor Xa but also prothrombinase complex and clot-associated factor Xa. Inhibition of factor Xa incorporated in the prothrombinase complex is a unique feature of small direct inhibitors and not a characteristic of antithrombin or antithrombin-dependent anticoagulants (Ageno et al. 2012). It is rapidly absorbed with high bioavailability and maximum plasma concentration is reached in 2–3 h. Its mean elimination half-life is 7–11 h in young healthy adults (11–13 h in the elderly). The effect of age, extreme body weight and gender on pharmacokinetic and pharmacodynamic properties of rivaroxaban has been evaluated. Findings suggest a single dose can be safely administered to all patients regardless of age, gender, or body weight. It is highly bound to plasma proteins in the range of 92–95 %, mostly albumin, and therefore it is not removable by hemodialysis. Elimination is about two-thirds via CYP3A4 and CYP2J2 and through CYP-independent mechanisms. About two-thirds are excreted by the kidneys; one-third of the active drug unchanged and one-third as inactive metabolites. The remaining third of the drug is metabolized by the liver and excreted in the feces. Drug interactions may occur with strong inhibitors for CYP3A4 and P-gp (i.e., ketoconazole and ritonavir). Rivaroxaban is not recommended in patients requiring treatment with azole-antimycotics (i.e., ketoconazole) or HIV protease inhibitors because of increased risk of bleeding. Macrolides (i.e., clarithromycin, erythromycin) are strong inhibitors of CYP3A4 and moderate P-gp inhibitors; however, they are not considered to have a clinically important interaction with rivaroxaban. Rifampicin is an inducer of CYP3A4 and P-gp and therefore will significantly decrease the effectiveness of rivaroxaban (Poulsen et al. 2012; Ageno et al. 2012).

Coagulation Assays

Rivaroxaban prolongs prothrombin time (PT), activated partial thromboplastin time (aPTT), and the *HEPTEST*. It does not affect bleeding time or platelet aggregation and has no effect on thrombin or antithrombin activity. Inhibition of factor Xa activity and PT prolongation correlates strongly with plasma concentrations in a linear fashion. These predictable properties are what allow for fixed dosing without the need for laboratory monitoring. However, there will be circumstances when measuring the anticoagulant effect is needed such as in cases of accidental overdose, bleeding, to assess compliance, to evaluate effect of drug interactions, and to evaluate for drug accumulation in patients with renal or liver impairment. Despite having predictable effects on multiple coagulation laboratory tests, there is presently no validated laboratory assay which can be used quantitatively to monitor rivaroxaban and there are no therapeutic targets or suggested dose adjustments recommended. The effect of rivaroxaban on the aPTT is weak and therefore the test is not suitable for quantitative monitoring. Standard methods for the HEPTEST, which measures factor Xa inhibition directly proportional to the concentration of heparin present, and prothrombinase-induced clotting time, sensitive to factor Xa and IIa inhibitors, gives paradoxical results. Assays used to measure anti-Xa activity for heparin and low-molecular-weight heparins show a dose-dependent but variable response (Samama et al. 2012). More validation and further study are needed to develop a reliable quantitative assay for clinical use (Ageno et al. 2012).

Reversal of Rivaroxaban

No specific antidote is yet available for reversal of the anticoagulant effects of rivaroxaban. In case of overdose or active bleeding the drug should be

discontinued and activated charcoal may be given to reduce absorption if the ingestion was recent. Dialysis is not likely to be helpful because of high plasma protein binding. Efficacy of using a particular hemostatic blood product has not yet been established. High dose four-factor PCC has been shown to normalize bleeding time in rats treated with intravenous rivaroxaban (Ageno et al. 2012) and to reverse its anticoagulant effect in healthy subjects (Eerenberg et al. 2011). Recombinant factor VIIa is suggested in cases of life-threatening bleeding however efficacy is not conclusively established (Ageno et al. 2012).

Apixaban

Apixaban is an oral direct inhibitor of factor Xa approved by the US FDA in January 2013 for prevention of stroke and systemic embolization in patients with non-valvular atrial fibrillation.

Key Clinical Trials

In a large randomized, double-blind noninferiority trial (ARISTOTLE) apixaban (5 mg twice daily) was compared to adjusted dose warfarin in 18,201 patients with atrial fibrillation with at least one additional risk factor for stroke. The primary outcome was ischemic or hemorrhagic stroke or systemic embolization. A secondary objective was to assess for superiority for primary outcome, major bleeding and death from any cause. Patients were followed for a median of 1.8 years with a significantly lower rate of the primary outcome (1.27 % per year with apixaban vs. 1.60 % per year with warfarin) demonstrating both noninferiority and superiority ($P<0.001$, $P=0.01$ respectively). Apixaban appeared to cause less bleeding and was associated with lower mortality compared to warfarin (Granger et al. 2011). In the AVERROES study, apixaban was compared to aspirin in patients with higher than average risk of thromboembolism and atrial fibrillation that were reluctant to take warfarin or thought to be unsuitable candidates. The study was double-dummy designed and 5,599 patients were randomized to either apixaban (5 mg twice daily) or aspirin (81–324 mg daily). The study terminated early because the primary end point of stroke or systemic embolism was 1.6 % in the apixaban group and 3.7 % in the aspirin group while rates of major bleeding and intracranial hemorrhage were similar (Connolly et al. 2011).

In the APPRAISE-2 study apixaban was assessed for the treatment of ACS. There was no significant difference in primary endpoint of cardiovascular death, MI or ischemic stroke in patients receiving standard therapy plus apixaban however there was an increased rate of major bleeding (1.3 % on apixaban vs. 0.5 % on placebo). The study was therefore terminated early (Alexander et al. 2011).

There have been three large double-blind, randomized, phase 3 clinical trials looking at apixaban for prophylaxis following major joint replacement surgery (ADVANCE-1-3). In the first study, 3,195 patients were randomized to either apixaban 2.5 mg twice daily or enoxaparin 30 mg subcutaneously injected every 12 h starting 12–24 h after total knee replacement surgery and continuing on for 10–14 days. The primary outcome defined as a composite of asymptomatic and symptomatic VTE and death from any cause during treatment was 9.0 % for the apixaban group and 8.8 % for the enoxaparin group. The rate of VTE in both groups was lower than expected which led to incorrect assumptions when determining the necessary sample size and criteria for calculating noninferiority. As a consequence, the criteria for noninferiority were not met for the primary outcome. However, the rate of major bleeding combined with clinically relevant nonmajor bleeding was 2.9 % in the apixaban group and 4.3 % in the enoxaparin group. Although apixaban did not meet the predetermined criteria for inferiority in this study, the authors conclude that it appears to have similar efficacy to enoxaparin with a lower risk for hemorrhagic complications (Lassen et al. 2009). In ADVANCE-2 apixaban 2.5 mg twice daily is again studied in total knee replacement surgery patients compared to enoxaparin with a slightly different dosing regimen. Enoxaparin was administered as a 40 mg subcutaneous injection once every 24 h beginning 12 h before surgery and

continuing for 10–14 days postoperatively. The primary endpoint was a composite of asymptomatic and symptomatic VTE and all cause death during treatment. A total of 3,057 patients were randomized to either treatment arm. The primary outcome occurred in 15 % of the apixaban group versus 24 % of the enoxaparin group translating to an absolute risk reduction of 9.3 %. Major or clinically relevant nonmajor bleeding occurred similarly in both groups. The authors conclude not only that apixaban is noninferior to this dosing regimen of enoxaparin, but that it is more effective for prevention of venous thromboembolism without increasing the risk of hemorrhagic complications (Lassen et al. 2010a, b). Total hip replacement surgery patients have also been studied using apixaban versus enoxaparin (ADVANCE-3). Apixaban 2.5 mg twice daily was compared with enoxaparin 40 mg once daily for thromboprophylaxis in patients following hip arthroplasty. This double-blind, double-dummy study enrolled 5,407 hip surgery patients. Thromboprophylaxis was extended to 35 days and the primary end point was a composite of asymptomatic or symptomatic DVT, nonfatal PE, or any-cause mortality. Apixaban met criteria for both noninferiority and superiority for the primary endpoint with similar rates of major bleeding (Lassen et al. 2010a, b).

In the ADOPT trial extended thromboprophylaxis with apixaban beyond hospital discharge was studied in medically ill hospitalized patients to determine if it could reduce the risk of VTE better and safer than using short term enoxaparin. In this double-blind, double-dummy, placebo controlled trial 4,995 patients were randomized and evaluated for the primary efficacy outcome. Eligible patients were hospitalized with congestive heart failure, respiratory failure, or other medical disorders with at least one additional risk factor for VTE and an expected hospitalization of at least 3 days. Patients were randomized to either apixaban 2.5 mg twice daily for 30 days or enoxaparin 40 mg once daily for 6–14 days. The primary efficacy outcome was a composite of death related to VTE, PE, symptomatic VTE, and asymptomatic proximal DVT at 30 days. Bleeding was the primary safety outcome. There was a 13 % reduction in the primary efficacy outcome in the apixaban group however the difference was not statistically significant (2.71 % in the apixaban group, 3.06 % in the enoxaparin group, $P = 0.44$). Major Bleeding was higher in the apixaban group (0.47 % vs. 0.19 %, $P = 0.04$). The authors concluded that extended thromboprophylaxis with apixaban was not superior to short course enoxaparin in medically ill patients and that apixaban was associated with more major bleeding events (Goldhaber et al. 2011). It should be noted that the rate of primary efficacy outcomes immediately increased once the enoxaparin injections were stopped and that extending thromboprophylaxis with enoxaparin beyond hospital discharge in medically ill patients is not usual medical practice. Therefore, there may in fact be a benefit of extending pharmacologic thromboprophylaxis in medically ill patients and further study and refinement of patient selection may clarify this in the future.

Apixaban is being studied for the treatment of VTE in an ongoing double-blind, phase 3 efficacy and safety clinical trial (AMPLIFY). It is being compared with enoxaparin to adjusted dose warfarin for a duration of 6 months for the treatment of acute VTE. A total of 4,816 patients are enrolled. The study is now closed to enrollment and final data collection results are not yet available. Apixaban has also been studied as an option for extended anticoagulant therapy for VTE in patients whom there was clinical equipoise in terms of continuing versus stopping anticoagulants (AMPLIFY-EX). The study was double-blinded and randomized 2,486 patients to receive either apixaban 2.5 mg twice daily or 5 mg twice daily compared to placebo for a duration of 12 months. All patients had already received a 6–12 month course of anticoagulation for the initial acute VTE. The primary endpoint of symptomatic recurrent VTE or death was much higher in the placebo group (8.8 %) as compared to either the prophylactic dose (1.7 %) or treatment dose (1.7 %) apixaban groups. There was no significant difference

in rates of major bleeding among all three groups however there was a trend toward increased clinically relevant nonmajor bleeding in the apixaban groups. The investigators concluded that extended anticoagulation with either prophylactic or treatment doses of apixaban results in a reduction of recurrent VTE while not significantly increasing the risk of major hemorrhage (Agnelli et al. 2013).

Pharmacokinetics

Like rivaroxaban, apixaban is an oral, direct, reversible inhibitor of factor Xa. It is absorbed rapidly and has no food interactions. The onset of action is 2–3 h and it is excreted 25 % by the kidneys and 75 % by the hepatobilliary route. The elimination half-life is 8–15 h in healthy adults. It is metabolized mostly by CYP3A4, CYP3A5, and sulfotransferase 1A1. Half of the dose is excreted unchanged in the feces. It is a substrate for P-gp. Anticoagulant effects are significantly increased by coadministration with strong inhibitors of both CYP3A4 and P-gp (i.e., ketoconazole, HIV protease inhibitors). Significant decreased plasma concentrations are seen with potent inducers of CYP3A4 and P-gp, most importantly rifampin. Phenytoin and carbamazepine are also strong inducers and would likely have a similar effect on plasma concentrations (Poulsen et al. 2012).

Coagulation Assays

Like the other oral directly inhibiting anticoagulants, apixaban does not require routine monitoring. Similar to rivaroxaban the PT, INR and aPTT are prolonged with apixaban, however the elevations seen in these clotting tests are often very small, even at therapeutic doses and variable results will be obtained depending on which reagent is used by a particular lab. This means a patient could be fully anticoagulated while their protime and aPTT are only slightly elevated. While not yet routinely available, an anti-Xa chromogenic assay calibrated using an apixaban standard has been shown to correlate with apixaban plasma concentrations (Rojas-Hernandez and Garcia 2013). There are no established therapeutic targets yet, but this test is a promising candidate for a simple and quick way to asses a patients level of anticoagulation while on factor Xa inhibitors.

Reversal of Apixaban

Similar to rivaroxaban, there is no antidote for reversal of the anticoagulant effect of apixaban. In the setting of bleeding or need for reversal the drug should be discontinued and activated charcoal can be used to reduce absorption of the medication if ingestion of the last dose was recent. The anticoagulant effect will last about 24 h after the last dose (two half-lives). It has high plasma protein binding therefore dialysis will likely have little effect on plasma concentrations. Protamine sulfate and vitamin K are not useful in reversal of anticoagulant effects from apixaban. Prothrombin complex concentrate, activated prothrombin complex concentrate and recombinant factor VIIa may be considered in the setting of life threatening bleeding however it has not been studied in clinical trials (source, prescribing highlights).

Future Oral Anticoagulants

There are numerous additional oral anticoagulants being studied at various stages of investigation. The most common target is factor Xa. Additional targets include thrombin, factor IXa, and factor XIa (Al-Horani et al. 2013). The most common indication being evaluated is for thromboprophylaxis after hip or knee replacement surgery. Additionally, factor Xa inhibitors betrixaban and eribaxaban are being studied for prevention of embolic complications of atrial fibrillation and letaxaban is being studied in patients with ACS (Ahrens et al. 2012) (Table 16.1). A summary of the features of the currently approved US FDA new anticoagulants is found in Table 16.1.

Table 16.1 Features of the new oral anticoagulants

	Dabigatran etexilate	Rivaroxaban	Apixaban
Target	Thrombin	Factor Xa	Factor Xa
Form	75 and 150 mg capsules	10, 15, 20 mg tablets	2.5 and 5 mg coated tablets
FDA approved indications	Non-valvular a-fib	Non-valvular a-fib, VTE prophylaxis after hip/knee replacement, treatment of VTE	Non-valvular a-fib
Dosing	150 mg BID if the CrCL is >30 ml/min and 75 mg BID if the CrCl is 15–30 ml/min	a-fib: 20 mg QD or 15 mg QD if CrCl is 15–50 ml/min VTE treatment: 15 mg BID × 21 days then 20 mg QD VTE prevention after hip/knee replacement: 10 mg QD	5 mg BID In patients with at least 2 of the following characteristics: age ≥80 year, body weight ≤60 kg, serum creatinine ≥1.5 mg/dL, the recommended dose is 2.5 mg orally BID
Bioavailability	3–7 %	~80 %	>50 %
Food	Delays absorption	No affect	No affect
Maximum concentration	1.5 to 3 h	2–3 h	3–4 h
Half-life	14–17 h	7–11 h	8–15 h
Elimination	80 % renal, 20 % liver	66 % renal, 33 % feces	~25 renal, 50 % biliary
Drug interactions: CYP/P-gp	No interaction with CYP, substrate for P-gp	3A4, 2J2, substrate for P-gp	3A4, substrate for P-gp
Renal impairment	CrCl < 30 reduce dose CrCl < 15 or on HD contraindicated	CrCl < 50 reduce dose CrCl < 30 or on HD contraindicated	CrCl < 15 or on HD contraindicated
Liver impairment	Contraindicated in severe liver impairment	Contraindicated in severe liver impairment	Contraindicated in severe liver impairment
Pregnancy/lactation	Insufficient data	Insufficient data	Insufficient data

FDA Food and Drug Administration, *a-fib* atrial fibrillation, *VTE* venous thromboembolic disease, *BID* twice daily, *QD* once daily, *CYP* cytochrome P450, *P-gp* P-glycoprotein

Discussion of Clinical Vignettes

Clinical Vignette 1 discussion: None of the new oral anticoagulants have been well studied in pregnancy or in lactating woman. They are all contraindicated in this setting.

Clinical Vignette 2 discussion: An advantage of the new oral anticoagulants is improved convenience and less complexity. However, they do not improve compliance with taking the medication. In the clinical setting of poor compliance, patients require close monitoring and more frequent visits to reinforce the need to take their medication. Dabigatran would not be a good option for this patient.

Clinical Vignette 3 discussion: The half-life of dabigatran in patients with normal to mildly impaired renal function is about 15 h thus for a standard risk elective surgery 24 h should be adequate for holding the drug. If bleeding risk is felt to be greater, then holding longer is advised (2–4 days). The most readily available and sensitive test to assess for the presence of direct thrombin inhibitor (DTI) activity is the thrombin time (TT). This test can be run 6–12 h prior to surgery and if elevated indicates DTI activity persists and the surgery should be postponed until it normalizes.

(continued)

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Pregnancy

17

Alan Lichtin and Elliot H. Philipson

Pregnancy

There are many reasons why a hematologist would be asked to see a woman who is (a) contemplating pregnancy but has a coagulation disorder or a family history of one, (b) pregnant and having either bleeding or thrombotic problems, (c) in labor and having a coagulopathy, or (d) in the postpartum period and having a coagulation concern. This chapter addresses the four issues separately, but begins by addressing the normal physiologic coagulation related issues that occur during a normal pregnancy and delivery.

Changes in Clotting Factors During Pregnancy (See Table 17.1)

During pregnancy, Factor VIII:C and FVIII von Willebrand factor (FVIII:VWF) levels rise steadily (Dossenbach-Glaninger et al. 2004), fibrinogen, factors VII and X also rise, but the levels of Factors II, IX, XII, and XIII stay about the same. Indeed, fibrinogen levels rise threefold (de Boer et al. 1989). Some studies of pregnant women show FXI level rising and some show a fall (Glueck et al. 2010). There are also changes in some of the naturally occurring anticoagulants. Protein C and antithrombin III levels stay constant, but Protein S levels fall (Faught et al. 1995). Fibrinolysis is reduced during pregnancy but can rise to normal within 1 h of delivery. Pregnancy induces an impairment of fibrinolysis through an increase in tissue plasminogen activator inhibitor-1 (PAI-1). Also, the placenta synthesizes plasminogen activator inhibitor-2 (PAI-2). Once the placenta is delivered, this substance's concentration falls rapidly and may be at a very low level within 1 h of delivery (Van Wersch and Ubachs 1991).

If one considers the logical needs of a pregnant woman as she approaches the birth process, it would make sense for the coagulation system to favor excessive clotting over excessive bleeding at the time of delivery. As the fetus is expelled, the placenta starts separating from the uterine wall, exposing a large surface of vessels which, for 9 months, were committed to O_2 exchange between maternal uterine arterioles and capillaries and the placental surface. As the uterus contracts after birth, there is physical tamponading of these vessels, but due to increases in clotting factor levels and decreases in fibrinolysis, the estimated blood loss from a normal delivery is usually about 500 cc (Sloan et al. 2010), and 1,000 cc for caesarean section.

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Table 17.1 Changes in coagulation factors during pregnancy

Factors that rise	Factors that stay the same	Factors that fall	Factors that rise and fall (or for which there are conflicting data)
Factor VIII:C	Factor II	Protein S	Factor V
Factor VIII: von Willebrand Factor	Factor IX	Plasminogen	Factor XI
Factor VII	Factor XII		
Factor X	Factor XIII		
Fibrinogen	Protein C		
Plasminogen activator inhibitors (PAI-1 and 2)	Antithrombin		

Women Contemplating Pregnancy Who Have Established Coagulation Abnormalities

A woman who knows that she has either an acquired or congenital coagulation disorder may present to the consulting hematologist seeking advice on how to handle that disorder during pregnancy and delivery. This section will deal separately with several of these disorders.

Bleeding Disorders During Pregnancy

von Willebrand's Disease

An extensive discussion of von Willebrand's Disease (VWD) is found in Chap. 4 of this book. Here, there will be a brief review of this disorder and then a longer discussion of the pregnancy and labor/delivery issues.

VWD is very common, affecting 2 % of the total population. Gradually, during the 9 months of gestation, levels of VWF protein at labor have risen three times as much as pre-pregnancy levels. VWD is divided into three types; type 1, type 2, and type 3. Type 1, which affects approximately 80 % of all those with VWD, usually has a minor effect on the pregnant woman. With the physiologic rise of FVIII:VWF levels through pregnancy, by the time the woman delivers, she may have normal VIII:VWF levels (Kujovich 2005). Women with type 2 or 3 VWD have no changes in the VIII levels, so they may require more attention to achieve hemostasis. Type 2A VWD is characterized by a more extensive fall of FVIII:Ristocetin Cofactor (FVIII:RCo) compared with FVIII:Antigen (FVIII:Ag) and lowered or absent levels of high and intermediate molecular weight VWF multimers. The lower the VIII:RCo, the more severe the bleeding. Type 2B VWD is caused by a genetically altered VIII:VWF molecule that has increased affinity for platelet membrane glycoprotein IB. As the VWF level rises during pregnancy, this can lead to a greater degree of thrombocytopenia. As platelets aggregate more, there may be a paradoxical increased risk of thrombosis. Type 3 VWD is characterized by very low levels of all VWF multimers and low levels of VIII:C, VIII:Ag, and VIII:RCo. These women experience no rise in their levels of these factors during pregnancy, and can have significant bleeding during pregnancy and during the postpartum period (Mannucci 2001).

If the consultant knows a woman has VWD and becomes pregnant, it is advisable to check FVIII levels every 2–3 months until the time of delivery, and continue checking up to 2 months postdelivery. The severity of bleeding complications correlates best with depth of VIII: RCo levels. Levels of 50 IU/dL (lower end of normal, normal being defined as roughly 50–150 IU/dL) are usually adequate for normal vaginal delivery or caesarean section. Levels below 50 IU/dL may require treatment with DDAVP (postpartum) or von Willebrand factor concentrates. Since VWF levels fall quickly after delivery, doses of DDAVP postpartum may help to prevent postpartum delayed hemorrhage (Lee et al. 2006). Other options include Humate P. If one looks at the package insert for Humate P, dosing is based on VIII:RCo units. A caesarean section should be considered a "major" surgical procedure,

and a normal vaginal delivery with episiotomy might be considered a little less risky, but it is not a "minor" procedure. Therefore, the dose of humate P prior to a planned or emergent C-section should be initiated with a loading dose of 50–75 IU/kg, and subsequently dosed at 40–60 IU/kg every 8–12 h for 3 days. It is imperative to maintain a nadir of 50 IU/dL of VIII:RCo. After 3 days, as long as the woman is not bleeding significantly, one may begin dosing every 24 h of 50 IU/kg (Lee et al. 2006).

DDAVP causes egress of VIII:C and VWF from Weidel Palade bodies in the endothelium. It is very useful in mild hemophilia and milder forms of Type I VWD to prevent bleeding after minor procedures. DDAVP does not cause uterine contractions, so may be used during pregnancy (Ray 1998), though most consultants wait until labor is well along before using it. If it is used daily, severe symptomatic hyponatremia can occur. Hypotonic saline should be avoided and women should be on fluid restriction. DDAVP should be avoided in Type 2B VWD because it exacerbates the thrombocytopenia.

Type 2N (Normandy) VWD

One of the subtypes of Type 2 VWD, the type 2N, is due to a genetic mutation affecting the site where VWF binds to the FVIII molecule. Multimer patterns are normal. FVIII:RCo and VIII:Ag levels are normal, but FVIII:C levels are very low—this condition can be easily confused with hemophilia A. Inheritance is autosomal recessive, however, and not X-linked recessive as in hemophilia A. These individuals do not have a good rise in FVIII level with infusions of FVIII:C, and should receive Humate P if their levels of FVIII:C are <50 IU/dL (Dennis et al. 2000).

Hemophilia (Factor VIII:C or Factor IX Deficiency)

Since hemophilia A (FVIII:C deficiency) and hemophilia B (FIX deficiency) are X-linked recessive disorders, women in these families are (often) carriers, and would be expected to have 50 % of normal levels. However, if there is severe Lyonization, the FVIII:C or IX level can be much lower. As these women approach labor, if their VIII:C or IX levels are <50 IU/dL, they may require DDAVP if VIII deficient or recombinant IX, if IX levels are <50 IU/dL. FIX levels do not rise during pregnancy like VIII:C does, so IX carriers may be more likely to bleed than VIII carriers.

FXI Deficiency

This deficiency status is common among Ashkenazi Jews. FXI is synthesized in the liver and deficiency is of autosomal recessive transmission. Levels below 20 % are associated with bleeding during surgery. Curiously, some individuals with severe deficiency (<1 % activity) do not bleed with surgery. Also, bleeding risk can vary over time, so a young person with homozygous deficiency (<1 %) may do fine with hernia repair at age 16, but can have severe bleeding during labor and the postpartum period.

There are conflicting data as to what happens to FXI levels during normal pregnancy. Some studies show an increase and some show a decrease (Martin-Salces et al. 2010). If a woman has less than 40 IU/dL at the time of labor/delivery, replacement with FFP is recommended (Myers et al. 2007; Lee et al. 2006). In Europe, there is a FXI concentrate available, but its use has been associated with thrombosis.

Uncommon Clotting Factor Deficiencies

Women who have FXIII deficiency have an elevated rate of miscarriage. To maintain pregnancy, these individuals need infusions of cryoprecipitate or FFP throughout their pregnancy and for up to 4 days postpartum (Burrows et al. 2000; Lee et al. 2006). Women who are hypofibrinogenemic or afibrinogenemic may also manifest difficulty maintaining pregnancy, and they too require infusions of cryoprecipitate. In women who are severely VII deficient, and who have a history of bleeding with surgery, the use of recombinant VII

A in the peripartum and for 3 days postpartum period at a dose of 15 μg/kg every 6 h is recommended (Burrows et al. 2000).

Platelet Disorders

There are several lifelong qualitative platelet disorders which can impact the outcome of pregnancy. Glanzmann's thrombasthenia is due to a deficiency of glycoprotein II B and III A (fibrinogen receptor) on the surface of platelets, which causes an inability for fibrinogen to serve as a bridge between platelets during aggregation. PT and PTT are normal, fibrinogen levels are normal, but platelet aggregation is flat line to all agonists. Platelet flow cytometry can document the severity of deficiency of II B/III A. This test is recommended to establish this diagnosis as well as to understand the severity of the bleeding risk.

Women with Glanzmann's thrombasthenia usually have menometrorrhagia, and bleeding can be severe and life threatening. Platelet transfusions are necessary to achieve hemostasis for surgery. Antifibrinolytics can help prevent hemorrhage with simple trauma (DiMinno et al. 2009).

If a woman with Glanzmann's thrombasthenia becomes pregnant, infusions of platelets during delivery, preferably single donor platelets, can help prevent severe bleeding. This should be done even if the patient has a normal platelet count. Postpartum hemorrhage can be abated with further platelet transfusion (Monte and Lyons 2002). There are case reports detailing the use of recombinant FVIIa in this setting. It is prudent to wait until after delivery to infuse this potent procoagulant because of concern for what it might do to compromise the placental circulation and the attendant effects on the fetus (Phillips et al. 2009; Poon et al. 2004).

Hermansky–Pudlak Syndrome

This syndrome is due to a combination of oculocutaneous albinism, a platelet storage pool defect, and a lysosomal storage accumulation of ceroid lipofusion. There are nine types and all are inherited as an autosomal recessive defect. These individuals usually have a mild bleeding diathesis, and this is best determined by taking a good history, but they typically exhibit pulmonary fibrosis around age 40, and that complication is the usual cause of death.

The genetic defect is a mutation of the HPS gene (many variants, e.g., HSP1, HSP3, HSP4). Each of these has been found on the long arm of chromosome 10. Some subtypes are seen in individuals of Puerto Rican ancestry and other subtypes are more common in Ashkenazi Jews.

Electron microscopy of platelets demonstrates an absence of dense bodies. Platelet aggregation studies demonstrate decreased platelet aggregation responses to ADP, arachidonic acid, collagen, and epinephrine with loss of secondary aggregation. The platelet aggregation to ADP at higher concentrations (20 μM) may normalize. The stimulated dense granule release responses to ADP (5 μM), arachidonic acid, collagen, and epinephrine are nearly absent. PFA-100 platelet function screen testing demonstrates prolongation of both col/epi and col/ADP cartridges.

Oftentimes platelet transfusions are necessary to achieve hemostases if the bleeding risk is higher, but for minor procedures, DDAVP may be all that is necessary, and this has been used for providing epidural analgesia during labor (Spencer and Rosengren 2009).

Bernard–Soulier Syndrome

This is a platelet disorder inherited as an autosomal recessive defect, which leads to a loss of, or less than normal amount of, glycoprotein IB on the platelet surface. This glycoprotein is the receptor for von Willebrand factor. The platelet count is low and the platelets are large. Bernard–Soulier platelets do not aggregate in the presence of ristocetin, and this defect is not corrected when normal plasma is added, a distinguishing feature between Bernard–Soulier syndrome and VMD.

Labor and delivery can proceed in women with this, but if the level of glycoprotein is very low, prophylactic platelet transfusion may be necessary. Again, platelets are the mainstay of treatment, but in milder cases, DDAVP and antifibrinolytics can help slow or prevent bleeding (Saade et al. 1991; Peaceman et al. 1989; Rahini et al. 2005).

Immune Thrombocytopenic Purpura

This is probably the most common and important consult for a hematologist who deals with pregnant patients. A woman who has a known diagnosis of immune thrombocytopenic purpura (ITP) may present a challenge to the obstetrician, hematologist, and pediatrician of the soon-to-be-born child. If she is only mildly affected and her platelet count is near normal, no special interventions may be needed. However, if her platelet count is very low, such as less than 20,000/μL, and since the antibodies mediating her platelet destruction can cross the placenta and affect the fetus' platelet count, the treating team may have two severely thrombocytopenic patients for whom they must care—the mother and the fetus (Gill and Kelton 2000).

A normal woman tends to drop her platelet count throughout pregnancy because of increased plasma volume (dilutional effect). This is called "gestational thrombocytopenia," and may be difficult to distinguish from mild ITP (Levy and Murphy 2002). If a woman does have a normal platelet count before pregnancy, then drops her platelets during pregnancy, and then platelets return to normal after pregnancy, and the baby is born with a normal platelet count, one may refer to her episode of thrombocytopenia as "gestational thrombocytopenia." Generally, gestational thrombocytopenia is defined as platelet counts between 100,000 and 150,000/μL. Often the platelets are a little larger than normal. Platelet counts between 70,000 and 100,000/μL may still be considered due to gestational thrombocytopenia, but the physician should assiduously rule out other causes of thrombocytopenia, such as HIV, hepatitis, lupus, and lupus anticoagulant positivity. This is a very important consideration. Admittedly, these tests are routinely done as soon as a woman is known to be pregnant, but it may be prudent to repeat some of these tests, as clinically indicated. It is also prudent to check the peripheral smear as the pregnancy proceeds, especially looking for schistocytes, and also making sure that white cell morphology is not changing, which can occur in a neoplastic process.

Women who drop their platelet count during pregnancy and raise their blood pressure, may be presenting with unique syndromes such as HELLP, preeclampsia, or DIC, and these will be discussed below.

The American Society of Hematology (ASH) published a clinical practice guideline on ITP in 1996 and it was updated in 2011 (Neunert et al. 2011). There is a section on ITP and pregnancy in the original guideline and, in the 2011 update, there was little change. Arbitrary platelet count levels of <10,000, 10,000–30,000, and >30,000 were used to guide decision making. If a woman has ITP and is pregnant, and her platelets are >30,000/μL and is not bleeding, nothing needs to be done except monitor her with periodic complete blood counts. If the platelet count is lower and there is bleeding, treatment is indicated. Both prednisone, at a dose of 1 mg/kg/day, and IVIgG, at a dose of 400 mg/kg daily for 5 days, or 1 g/kg daily for 2 days were considered appropriate and safe for both the woman and the fetus. There was commentary and caution in the guidelines about steroid induced diabetes and depression. It was recommended to avoid cyclophosphamide and vinca alkaloids because of teratogenicity. Splenectomy was not encouraged during pregnancy because of increased risk of preterm labor during first trimester, and it is technically difficult to do in the third trimester (Felbinger et al. 2007).

There is a separate section on "Treatment of ITP during labor & delivery" in the guidelines (Neunert et al. 2011). Measurement of fetal platelet count can be achieved with percutaneous umbilical blood sampling (PUBS), but that was not encouraged due to risks, especially fetal demise. Fetal scalp puncture/sampling can only occur once labor has commenced and the fetal head is already well down the birth canal. Ultimately, the guideline recommends that "ITP management during labor and delivery is based on an assessment of maternal bleeding risks associated with epidural anesthesia and with delivery and the minimum platelet counts required to

safely undergo these procedures" (Neunert et al. 2011). There was no evidence to support specific platelet count thresholds that are safe in the anteor peripartum period. In a single center retrospective review of labor management of 119 mothers with ITP, epidural anesthesia was given for a maternal platelet count <50 K in one case, and 50–75,000 in six cases. Vaginal delivery was carried out in 82 % of deliveries and in 18 %, C-sections were performed. One quarter (25 %) of the neonates were born with thrombocytopenia, but there were only rare events of severe bleeding (Webert et al. 2003). Caution should note that this was a Canadian study, and the litigious climate in the USA would likely sway a treating obstetrician to perform C-sections more frequently in mothers with ITP.

Management of the baby, once born, to a mother with ITP necessitates careful monitoring of the platelet count. Platelets may fall during the first week of life. IVIgG is generally given to the baby to maintain a safe platelet count, until the maternal antibodies mediating the fetus' thrombocytopenia have cleared.

Thrombotic Disorders During Pregnancy (See Table 17.2)

This part of the chapter will be divided into two parts. The first section will deal with those individuals with known underlying thrombotic disease who are contemplating pregnancy, and the second part will discuss those women who become pregnant and develop an unexpected thrombotic disorder during pregnancy or postpartum.

Table 17.2 Venous thromboembolism (VTE) in pregnancy

Pregnant women have a fourfold to fivefold increased risk of VTE compared with nonpregnant women
80 % of VTE events in pregnancy are venous with a prevalence of 0.5–2.0 per 1,000 pregnant women
VTE accounts for 1.1 deaths per 100,000 deliveries, or 9 % of all maternal deaths in the USA
75–80 % of VTE are deep vein thromboses (DVT), and 20–25 % are due to pulmonary embolism (PE). One half of these events occur during pregnancy and one half occur during the postpartum period

Thrombophilic Women Before Pregnancy (See Tables 17.3 and 17.4)

It is not uncommon for a woman with a known hypercoagulable state to come to her physician questioning her risks if she were to become pregnant. These women may be completely asymptomatic, and have been tested for thrombophilia because of a family member who was identified as having a thrombophilic disorder. They may be completely asymptomatic at the time of the consult, but they may have had a previous manifestation of venous thromboembolic disease. They may have never been pregnant before, or they might have been pregnant and had a miscarriage, or even may have had a completely normal pregnancy and delivery in the past.

Pregnancy is a prothrombic state for many reasons. There are clotting factor changes with elevations of procoagulant factor levels (de Boer et al. 1989; Glueck et al. 2010) and decreases of endogenous anticoagulant factors (Faught et al. 1995), such as Protein S, impaired fibrinolysis (Van Wersch and Ubachs 1991), and an acquired resistance to the action of activated Protein C. There is venous stasis and hormonally driven increases in the distensibility of the veins in the legs and pelvis. Also, the gravid uterus sits directly on the veins draining the legs and by physical force tends to slow down the flow of blood out of the legs.

Table 17.3 Indications for thrombophilia testing prior to or during pregnancy[a]

Testing recommended
Personal history of thrombosis
First-degree relative with known thrombophilia and history of thrombosis
Testing should be considered
Family history of thrombophilia
Prior unexplained fetal death associated with abnormal placental pathology
Prior severe onset preeclampsia (<34 weeks gestation)
Prior severe intrauterine growth retardation (IUGR)

[a]Testing is more likely to be helpful for thrombophilias with high risk for thrombosis (e.g., antithrombin deficiency) rather than Factor V Leiden

Table 17.4 Thrombophilia screening tests in pregnancy[a]

Factor V Leiden
Prothrombin gene mutation
Antithrombin deficiency
Deficiency of Protein S and C[b]
Anticardiolipin antibodies and lupus anticoagulants
Cutoff value for free protein S antigen in the second trimester <30 % Cutoff value for free protein S antigen in the third trimester is <24 % [a]Screening for MTHFR is not indicated [b]Do not screen for Protein S during pregnancy or while on oral contraceptives

Table 17.5 Venous thromboembolism (VTE) prophylaxis in pregnancy and postpartum

Consultation with maternal–fetal medicine is recommended for:
Patient diagnosed with high-risk thrombophilia
Patient with prior VTE event
Patient taking long-term anticoagulation therapy prior to pregnancy
Patient with valvular heart disease or arrhythmia
Patient started on anticoagulation therapy during this pregnancy by outside physician without clear indication
Patients should be informed of the risks, benefits, side effects, and alternatives to anticoagulation medication
Complicated cases may require consultation with vascular medicine or hematology-oncology to determine an individualized management plan
Patients requiring long-term anticoagulation should be referred to a coumadin clinic after delivery

Table 17.6 Classification of thrombophilia (obstetric viewpoint)

High-risk thrombophilias
Factor V Leiden (FVL) homozygous
Prothrombin Gene (PTG) mutation homozygous
FVL heterozygous and PTG heterozygous
Antithrombin deficiency
Low-risk thrombophilias
FVL heterozygous
PTG mutation heterozygous
Protein C deficiency
Protein S deficiency

Maternal deep vein thromboses and pulmonary emboli occur in about 0.05–0.2 % of all births. Pulmonary embolism is a major cause of maternal death (Dresang et al. 2008). Thrombophilic states also interfere with normal placental function and can lead to miscarriage, especially due to antiphospholipid antibodies (Derksen et al. 2001). This acquired autoimmune disorder may be a reason why a woman might have given birth normally, at first, and then once acquiring the thrombophilic defect, begin to have miscarriages.

The most common thrombophilic defects that are examined in the laboratory now are (1) Factor V Leiden, (2) prothrombin gene 20210 mutation, (3) lupus anticoagulant, and (4) presence of a myeloproliferative state. By far, the more common reasons for a clinically driven risk for VTE are smoking, obesity, family history of VTE, and a sedentary lifestyle (see Tables 17.5 and 17.6).

If a woman has a history of being on oral contraceptives for years without developing a VTE, the chance that an underlying congenital thrombophilic state like homozygous (more risk) or heterozygous (less risk) FV Leiden will cause VTE during pregnancy, is lower (Dizon-Townson et al. 2005). If she had a fractured leg, and did not develop a VTE while in a cast for several weeks, that too is a soft finding favoring that she would not develop a VTE with pregnancy.

Homozygosity for genetic defects like FV Leiden (Heit et al. 2005) and prothrombin gene mutation (Gerhardt et al. 2000) is obviously worse than heterozygosity. However, if a mother is homozygous for the MTHFR mutation without a rise in homocysteine level, there is no increased risk for VTE (Varga et al. 2005).

Some women have more than one thrombophilic state, and there are synergistic effects when more than one thrombophilic state is present. As stated in the "Thrombotic Risk Factors" chapter of this text, heterozygosity for Factor V Leiden or prothrombin gene 20210 mutation confers only a small increased risk for VTE. However, if there is combined heterozygosity, the risk is greater. A pooled analysis of case–control studies found odds ratios for VTE of 4.9 and 3.8 for Factor V Leiden and prothrombin G20210A mutations, respectively, but an odds ratio of 20 in double heterozygotes (Emmerich 2001; see this reference in Price and Minichiello's chapter in this text).

On the other hand, if one screens the general population of pregnant women, without a causative question arising during a normal OB/patient relationship, very low rates of VTE are demonstrated. In one study (Lindqvist et al. 1999), there

was no case of VTE during pregnancy or during the postpartum period in a large group of women found to be heterozygous for FV Leiden by general screening. It is for this reason that screening for thrombophilic states is *NOT* recommended.

Antithrombin deficiency carries an even higher risk than the other commonly tested congenital thrombophilias (Yamada et al. 2001). Depending upon how one defines the level of an antithrombin deficiency, rates of 1 in 3, or up to 1 in 250 pregnancies may be associated with VTE.

If a woman is known to be antithrombin deficient and has been receiving LMWH to prevent clotting during pregnancy, one can administer exogenous recombinant antithrombin concentrates. These can be given at a dose of 50 U/kg prior to delivery. One should aim to keep the plasma antithrombin level near 100 % activity until the time is appropriate to reinitiate LMWH after the delivery. One may need to give this daily for up to 7 days (Hellgren et al. 1982; Schulman and Tengborn 1992).

How to Diagnose DVT/PE in a Pregnant Woman?

A woman who is pregnant and comes to the doctor with shortness of breath, chest pain, hemoptysis, unexplained hypoxia, or a painful or swollen leg should be tested for suspected PE and/or DVT. Sometimes, it is very hard to know when to pursue this workup, because many normal pregnant women have swollen legs and can be short of breath from carrying the extra weight. The testing used should have the lowest possible radiation exposure to the fetus. Which trimester the woman is in makes this question more imperative because the fetus is more sensitive for teratogenicity with radiation exposure in the earliest phases of pregnancy, i.e., first trimester, and can tolerate this potential insult much better closer to term (Winer-Muram et al. 2002).

Because of anatomic considerations, many more DVT's occur in the left leg than in the right (Scarsbrook et al. 2006). This is because the right internal iliac artery is in such close proximity to the left iliac vein. During pregnancy, the gravid uterus pushes back over this area and compresses the left iliac vein more than the right. In some series, the left leg is the site of DVT 85 % of the time (Ginsberg et al. 1992). This phenomenon is often referred to as the May–Thurner Syndrome.

Doppler venous ultrasound of the leg is the most helpful tool in establishing a DVT diagnosis. It is noninvasive and uses no radiation. It is highly sensitive and specific. It is a little less reliable in a woman who has a distant history of DVT, because the technician performing the study, and the ultrasonographer reading the scan, may have difficulty distinguishing an old clot from a new clot. In some centers, D-dimer is a test drawn to aid in deciding if a clot is present. This test is more unreliable during pregnancy because the D-dimer level rises, usually above the normal range, as a normal pregnancy progresses (Kline et al. 2005).

When a vein is affected by DVT in the more proximal locations (closer to the inferior vena cava, or IVC, or external iliac vein), the Doppler venous ultrasound may be less helpful to establish a diagnosis. Some centers use magnetic resonance venography without gadolinium to establish an IVC or iliac vein thrombosis diagnosis (Fraser et al. 2002). Gadolinium contrast is to be avoided during pregnancy because this agent freely crosses the placenta. When excreted by the fetal kidneys, it stays in the amniotic fluid until birth. It has the potential to cause toxic side effects to the fetus/infant.

Calf vein DVT's are not as common as one would think during pregnancy, occurring in around 8 % of women who develop a DVT during pregnancy. Many of these spontaneously involute, so serial Doppler studies are helpful in determining whether extension occurs into the more proximal veins, necessitating anticoagulant therapy.

For pregnant women with suspected pulmonary embolism, it is important for them to undergo proper radiographic testing, especially CT pulmonary angiography. Reducing the amount of radiation to the woman and the fetus can be achieved, and still get an acceptable image. There are national radiologic and nuclear medicine guidelines on how to reduce the radiation dose, as well

as IV contrast dose for pregnant women (Pahade et al. 2009). Ventilation perfusion scanning also can be accomplished with use of lower doses of radiation. It is also important for all radiology departments to identify whether a female patient is pregnant. Documentation of discussions with pregnant patients about radiation to be received, and putting this documentation in the radiology report, may mitigate the medicolegal risk (Pahade et al. 2009).

What Anticoagulant to Use? (See Tables 17.7, 17.8 and 17.9)

If a woman is having a DVT or PE, or if a woman with a defined increased risk for miscarriage or DVT/PE is identified and a decision has been made she needs anticoagulation, there are several choices of medication to use. If there are delays in testing for DVT/PE either because of being in a small facility without adequate radiographic equipment, or it is the middle of the night, or staff are not available, it is recommended to anticoagulate first, and then work on diagnosis later. Coumadin is contraindicated during pregnancy because it harms the fetus (Vitale et al. 1999). There is some debate about this, but the preponderance of literature recommends against using coumadin during pregnancy. Coumadin's teratogenicity is greatest during the first trimester when limb buds are forming. Maternal ingestion of coumadin affects the coagulation status of the fetus. Bleeding into the developing limb buds causes stippled epiphyses (thus shortened limbs), cerebral hemorrhage, and underdevelopment of the nose.

It is much preferred to use an agent that does not cross the placenta. There is the most experience using unfractionated heparin (UFH) and low molecular weight heparin (LMWH). There is a greater risk of osteoporosis and heparin-induced thrombocytopenia with UFH, so LMWH is the preferred choice (Greer and Hunt 2005; Greer and Nelson-Piercy 2005). Some women develop allergy to LMWH (for example, hypersensitivity skin reactions), and alternatives for it include direct thrombin inhibitors (leprudin and argatroban), or fondaparinux. The latter agent binds to antithrombin and potentiates antithrombin-mediated inactivation of factor Xa. It is a pentasaccharide, and because of its relatively small size, it does cross the placenta, but to a low level (Dempfle 2004). It does not cross react with HIT antibodies, so it can be used in the rare woman who develops HIT during pregnancy.

If LMWH is used during pregnancy, it is best to monitor factor Xa levels. FVIII levels rise during pregnancy and makes the PTT unreliable for assessing true anticoagulant activity for heparin. Anti-factor Xa levels of 0.3–0.7 IU/mL are considered therapeutic for heparin. For LMWH, peak levels are measured 4 h post-injection and trough levels are monitored just prior to a next injection (trough level). The target anti-Xa level

Table 17.7 Anticoagulation regimens for pregnancy and the postpartum

Prophylactic low-molecular-weight heparin (LMWH)[a]
Enoxaparin 40 mg subq once daily
Therapeutic LMWH
Enoxaparin 1 mg/kg every 12 h[b]
Minidose prophylactic unfractionated heparin (UFH)
UFH 5,000 U subq every 12 h
Prophylactic UFH
UFH 5,000–7,500 U subq every 12 h in first trimester
UFH 7,500–10,000 U subq every 12 h in the second trimester
UFH 10,000 U subq every 12 h in the third trimester, unless aPTT is elevated
Therapeutic UFH (also referred to as weight adjusted, full treatment dose)
UFH 10,000 U or more subq every 12 h in doses adjusted to target aPTT in the therapeutic range (1.5–2.5 times control) 6 h after injection
Postpartum anticoagulation
Prophylactic LMWH/UFH for 6 weeks or vitamin K antagonists for 6 weeks with a target INR of 2.0–3.0, with initial UFH or LMWH therapy overlap until the INR is 2.0 or more for 2 days
Surveillance
Clinical vigilance and appropriate objective investigation of women with symptoms suspicious for DVT or PE

[a]Although at extremes of body weight, modification of dose may be required

[b]May target an anti-Xa level in the therapeutic range of 0.5–1.0 U/mL for twice daily regimen; slightly higher doses may be needed for a once-daily regimen

Table 17.8 Recommended thromboprophylaxis for pregnancies complicated by low-risk inherited thrombophilia

Scenario	Antepartum	Postpartum
Low-risk thrombophilia without previous VTE	Surveillance without anticoagulation therapy or prophylactic LMWH or UFH	Surveillance without anticoagulant therapy or postpartum anticoagulation
Low-risk thrombophilia with a single previous episode of VTE—not receiving long-term anticoagulation	Prophylactic or intermediate dose LMWH/UFH or surveillance without anticoagulation	Postpartum anticoagulation therapy or intermediate dose LMWH/UFH

Table 17.9 Recommended thromboprophylaxis for pregnancies complicated by high-risk inherited thrombophilia

Scenario	Antepartum	Postpartum
High-risk thrombophilia without previous VTE	Prophylactic LMWH or UFH	Postpartum anticoagulant therapy
High-risk thrombophilia with a single previous episode of VTE—not receiving long-term anticoagulation	Prophylactic, intermediate dose, or adjusted dose LMWH/UFH	Postpartum anticoagulation therapy or intermediate or adjusted-dose LMWH/UFH for 6 weeks (therapy level should be at least as high as antepartum treatment)

for LMWH is generally 0.5–1.0 IU/mL (Greer and Hunt 2005; Greer and Nelson-Piercy 2005).

An acute proximal DVT or PE during pregnancy necessitates full dose therapeutic doses of anticoagulation. LMWH is probably the preferred treatment. LMWH should be given twice per day at a dose of 1 mg/kg subcutaneously (subq) or dalteperin 100 u/kg subq every 12 h. When the decision is made to use UFH, a pre-heparin PTT is obtained, and an IV bolus is given, usually 80 U/kg bolus followed by 18 U/kg/h to maintain an anti-Xa level of 0.3–0.7 IU/mL for heparin. After 5 days of IV UFH, the pregnant woman is converted over to full dose subcutaneous UFH or LMWH at therapeutic doses for the remainder of the pregnancy and for 6 weeks after delivery.

As the time of delivery approaches, one must hold the UFH or LMWH. If a planned C-section occurs, one stops the LMWH 24 h prior to delivery. Epidural catheters should not be placed unless the last LMWH dose was given at least 12 h before. One should not restart LMWH until at least 2–4 h after the catheter is removed (Meslovitz et al. 2005). There have been many case reports of epidural hemorrhage and irreversible neurologic sequelae when LMWH is used too close in time to the insertion or removal of epidural catheters (Wysowski et al. 1998).

If delivery is not a planned C-section, mothers should be alerted to stop LMWH at the onset of labor pains. One can switch to unfractionated heparin at 36 weeks. If labor is precipitous, and there is still a likelihood of circulating LMWH still present, one should avoid placing an epidural. Protamine sulfate does not reverse the full anticoagulant effect of LMWH. It only reverses about 60 %. If a woman on LMWH has a prolonged labor, and is at very high risk for recurrent VTE, one can use IV UFH to bridge the woman, stopping 6 h prior to delivery (Gibson 2009).

After the baby is born, one should be certain hemostasis is appropriate before reinitiating any anticoagulants. Inspection of episiotomy or C-section incisions must demonstrate no ongoing bleeding before one can be satisfied that it is safe and prudent to start giving either UFH or LMWH again. One may also start coumadin soon after UFH or LMWH has been restarted. It is wise not to start coumadin first, to avoid the dreaded complication of warfarin-induced skin necrosis (Chan et al. 2000). One should not stop the UFH or LMWH until the INR has reached a therapeutic level after a minimum 5 day overlap. This is usually 2.0, unless the mother has lupus anticoagulant, in which case a therapeutic INR might be much higher (Bijsterveld et al. 2000). In that

setting, a therapeutic INR is that INR that occurs when the factors II and X levels are at 20 % or below. That INR level might be 3 or 4, or even potentially higher (Osinbowale et al. 2009). The duration of anticoagulation depends upon when in pregnancy the VTE occurred, the underlying congenital or acquired risk factors for thrombophilia, and other coexisting risk factors, like smoking or obesity (Prandoni et al. 2007). In an uncomplicated VTE with minimal or no risk factors, and it was just the pregnancy itself that tipped the balance to thrombosis, postpartum anticoagulation should continue for 6 weeks, or for a minimum of 3 months total duration, if the VTE occurred late in pregnancy (White et al. 2008; Prandoni et al. 2011; Bates et al. 2012). If the woman has antithrombin deficiency or lupus anticoagulant, long-term anticoagulation is mandated. It is safe to breast feed the infant if the woman is receiving postpartum anticoagulation. Coumadin, UFH, LMWH, and fondaparinux do not enter the breast milk (Schindler and Graham 2011).

If a pregnant woman cannot tolerate any anticoagulation for a DVT or PE, then an IVC filter should be placed. These might be pregnant women with GI bleeding (non-modifiable sources of GI bleeding like hereditary hemorrhagic telangiestasia, or Osler–Weber–Rendu syndrome), or cerebral aneurysms. If a woman has recurrent VTE despite therapeutic anticoagulation, that is an absolute indication to use an IVC filter. This is probably the most common reason for a pregnant woman to require an IVC filter. Even after a filter is placed, if she can tolerate it, further anticoagulation should be administered. There are more data accumulated demonstrating that retrievable filters work well in pregnancy, and they can be removed at least 6 weeks after delivery, if appropriate (Kawamata et al. 2005).

From the interventional radiologist's perspective (or whichever person is inserting the filter), jugular access for insertion of a filter is preferred. Another option would be a shielded pelvis (wrapped in a lead apron to protect the fetus). The preferred site to leave the IVC filter is in a suprarenal location. However, depending upon body habitus, infrarenal location is an option. A retrievable and/or repositional designed filter is also preferred as opposed to a permanent one.

Massive PE's that threaten the life of the mother have been treated with thrombolytic agents (Leonhardt et al. 2006). The risks of severe bleeding in pregnancy are the same as nonpregnant patients, at about 6 %. Fetal loss has occurred during thrombolytic therapy. Other indications besides large PE's, would include acute stroke, clots on heart valves, and particularly large DVT's affecting the ileofemoral veins (Leonhardt et al. 2006).

Anti-Phospholipid Antibodies

Anti-phospholipid antibodies (APLA) are autoantibodies directed against cell membrane phospholipids, which cause excessive blood clotting on both the arterial and venous sides of the circulation. Anti-phospholipid antibodies are a collection of antibodies directed against cardiolipin and β-2-glycoprotein. They are discussed in detail in the chapter on workup of the prolonged PTT. Indeed, these antiphospholipid antibodies are usually detected because the PTT is prolonged, and on inhibitor screening, there is immediate inhibition of the PTT test, as opposed to delayed inhibition as occurs with clotting factor inhibitors.

Pregnant individuals with anti-phospholipid antibodies suffer recurrent pregnancy loss (RPL), still births, preterm delivery, and preeclampsia. Some authors define the adverse pregnancy outcomes of anti-phospholipid antibodies to include three or more spontaneous miscarriages before 10 weeks gestation, one or more unexplained spontaneous miscarriage after 10 weeks, and premature birth (before 34 weeks) due to placental insufficiency or severe preeclampsia (Wilson et al. 1999; Miyakis et al. 2006). Some women with anti-phospholipid antibodies have VTE, in addition to RPL, and some just have RPL. There is a temporal importance to the occurrence of anti-phospholipid antibodies, because they should be documented on at least two separate occasions 12 weeks apart.

By just screening otherwise healthy pregnant women for anti-phospholipid antibodies, one

would find anywhere from 2 to 9 % positive. Therefore, it is recommended not to screen women who do not have a suggestive history. This variation depends on definitions of cutoffs for deciding when a woman is truly positive. If one screens women with RPL, one finds up to 20 % positive for APLA's. When one screens women who have pregnancy associated VTE, one may find up to 27 % positive for APLA's (Derksen et al. 2004).

If a woman with known APLA's is on coumadin for VTE and is contemplating pregnancy, she must be switched over to UFH or LMWH, and then conceive. At no time of the pregnancy should she be taking coumadin (see the debatable aspect of this above). If a woman has APLA's and has no history of thrombosis and is just being observed, it is advisable for her to start full dose UFH or LMWH once she knows she is pregnant. This is common obstetric practice, but the updated CHEST guidelines do not specifically comment on this (Bates et al. 2012). This should help prevent the risks to the fetus of this procoagulant state. Some authors might advise aspirin (Tincani et al. 2003) as opposed to UFH or LMWH if the titers of the APLA are low, and some advocate low-dose aspirin and prophylactic dose of UFH and LMWH. Once an asymptomatic APLA patient delivers, 6 weeks of postpartum coumadin is advised (again, after at least 5 days overlap UFH or LMWH so as to avoid warfarin-induced skin necrosis).

The current CHEST guidelines comment that when confronted with a woman with APLA's and three or more pregnancy losses, or a late pregnancy loss and no history of venous or arterial thrombosis, one should administer antepartum prophylactic or intermediate dose UFH or prophylactic LMWH combined with aspirin (Bates et al. 2012, and see Table 17.7).

If a woman has APLA plus other risk factors for VTE, such as FV Leiden, prothrombin gene mutation, low protein C or S, or hyperhomocysteinemia, and has a previous history of pregnancy loss or VTE, low-dose aspirin plus adjusted dose UFH or LMWH is advised. This is a common obstetric practice but, again, is not specifically commented upon in the current CHEST guideline.

Clinical Vignette 1: Neonatal Alloimmune Thrombocytopenia

A 34-year-old G3P2 female, currently 11 weeks pregnant, is referred to you for evaluation of neonatal alloimmune thrombocytopenia (NAIT). Her first pregnancy was without complications. However, she states that during her second pregnancy in September 2008, she was diagnosed with gestational diabetes and underwent induction at 39 weeks. Due to issues with bradycardia and fetal distress, she underwent emergency Caesarian section. She states that she noted scattered petechiae diffusely on the newborn baby. The following day, during lab testing, it was noted that the baby was oozing blood with excessive bleeding. This prompted a CBC which revealed a platelet count of 41,000/μl. The baby was treated with IVIgG on the same day, and repeat platelet count the following day demonstrated a rise to 142,000/μl. A repeat platelet count on the baby 1 week later was 395,000/μl. The baby did not have any further episodes of bleeding and is now 5 years old.

The woman is now pregnant with a third child. She and her partner underwent an evaluation which demonstrated that she is homozygous HPA-1b and has anti-HPA1a antibody, and the father of the baby is HPA-1a homozygous. The mother's platelet count is 244,000/μl.

Further analysis is as follows (Table 17.10):

Strong positive reactions detected in the mother's serum against HPA-1a positive platelets only. The platelet typing studies with the serologic results support a diagnosis of Neonatal Alloimmune Thrombocytopenia (NAIT) due to an incompatibility for HPA-1a in this family. Since the father is homozygous for the HPA-1a, subsequent pregnancies in this family are at extremely high risk (approaching 100 %) of being affected with NAIT. In the event future pregnancies

(continued)

Table 17.10 Platelet antibody identification

(a) Antigen capture ELISA II							
Class I HLA			*Pool Ib/IX*			*Pool IV*	
Negative			Negative			Negative	
(b) Modified antigen capture ELISA							
GPIIb/IIIa			*HPA 1a/1a–3a/3a*			*HPA 1b/1b–3b/3b*	
			Positive			Negative	
GPIa/IIa			*HPA 5a/5a*			*HPA 5b/5b*	
			Negative			Negative	
(c) Modified antigen capture ELISA crossmatch							
Father—IIb/IIIa							
Positive							
(d) Platelet antibody screen							
			IgG result			*IgM result*	
Target platelet 1			Positive			Negative	
Target platelet 2			Negative			Negative	
Mother's platelets			Negative			Negative	
Father's platelets			Positive			Negative	
(e) Platelet antigen typing—mother							
HPA-1	*HPA-2*	*HPA-3*	*HPA-4*	*HPA-5*	*HPA-6*	*HPA-9w*	*HPA-15*
HPA 1b/1a	HPA 2a/2b	HPA 3a/3b	HPA 4a/4a	HPA 5a/5a	HPA 6a/6a	HPA9a/9a	HPA 15a/15b
(f) Platelet antigen typing—father							
HPA-1	*HPA-2*	*HPA-3*	*HPA-4*	*HPA-5*	*HPA-6*	*HPA-9w*	*HPA-15*
HPA 1a/1a	HPA 2a/2a	HPA 3a/3b	HPA 4a/4a	HPA 5a/5a	HPA 6a/6a	HPA9a/9a	HPA 15a/15b

are contemplated, genetic counseling would be appropriate.

The genotype was determined from genomic DNA using PCR and fluorescent hydrolysis probes specific to the a and b alleles of the Human Platelet Antigen systems 1–6, 9, and 15. Analytical sensitivity is >99 %. Rare polymorphisms within primer or probe regions may interfere with detection of gene variants (Blood Center of Wisconsin).

Neonatal Alloimmune Thrombocytopenia

Neonatal alloimmune thrombocytopenia (NAIT) is that condition in which maternal antibodies against fetal platelet antigens cross the placenta and lead to premature destruction of fetal platelets. The baby is born with severe thrombocytopenia and can have ominous consequences, especially intracranial hemorrhage (ICH). NAIT occurs in 1:1,000 pregnancies. Severe NAIT is defined as a platelet count at birth of less than 50,000/μl.

Human platelet alloantibodies occur when a human platelet antigen (HPA) is found on the fetus' platelets but not on the mother's platelets. NAIT can occur in a primagravida, but more typically occurs in the second, third, or subsequent pregnancy.

In Caucasians, the two most important HPA's are HPA-1a and HPA-5b or the HPA-1 and HPA-5 systems. HPA alloantibodies can be either of the IgG or IgM class, but only the IgG can cross the placenta. One cannot predict, by laboratory testing, how low a neonate's platelet count will drop, though the severity of previous pregnancy outcomes can give one a sense of what might occur with subsequent pregnancies. If the father is heterozygous for the relevant HPA antigen, there is a 50 % chance that his child will be negative. If he is homozygous, there is almost certainty that the child will be born thrombocytopenic. One can determine the fetal HPA group in the first trimester using DNA amplification techniques of cells derived from amniocentesis from as early as 14 weeks gestation.

Babies affected by NAIT are born with petechiae and ecchymoses. ICH is rare, fortunately. The treating neonatologist must rule out other reasons for severe thrombocytopenia, such as disseminated intravascular coagulation (DIC), infections, or congenital anomalies. In a baby with NAIT, the platelet count can fall from the day of birth and nadir at Day 3–4 of life. Random donor platelet transfusions may help raise the platelet count, but antigen negative single donor platelets are preferred. The usual treatment involves giving the baby IVIgG until the platelet count improves. Once a woman is found to be at risk for delivering an infant with NAIT, she is treated with weekly IVIgG starting at 20 weeks gestation, at a dose of 1 g/kg. Steroids may also be of benefit.

Clinical Vignette 1 (Continued)
The mother starts weekly IVIgG at a dose of 1 g/kg weekly at 20 weeks gestation. Since she had gestational diabetes with her second child, the mother is reluctant to take steroids, though is convinced to take a low dose (10 mg daily), starting at 36 weeks.

She delivers a healthy baby, whose platelet count at birth is 210,000/μl.

Problems That Occur in Women for Which a Hematologist Will Be Consulted Later in Pregnancy

HELLP Syndrome, Preeclampsia, Eclampsia

Long awaited updated guidelines about the hypertensive disorders of pregnancy have just been published (Roberts et al. 2013). This document has new classifications of four types of hypertension in pregnancy (one of which is preeclampsia/eclampsia). The older definition of preeclampsia/eclampsia included proteinuria, which is not necessary according to these new guidelines. Therefore, physicians should not wait for the occurrence of proteinuria before invoking preeclampsia as a diagnosis – waiting for proteinuria can delay diagnosis and treatment. These three syndromes represent a spectrum of hypertensive disorders that occur during the last half of pregnancy, or even postpartum. In HELLP syndrome (Weinstein 1982), *h*emolysis, *e*levated *l*iver function tests, and *l*ow *p*latelets occur. There are many different scoring systems and criteria for labeling a woman with HELLP syndrome. The peripheral smear (see Fig. 17.1) must show an element of microangiopathic hemolytic anemia, platelets <100,000 μL, LDH >600 mg/dL, and indirect hyper-bilirubinemia and elevated SGOT (>70 μ/l) are seen on lab studies (U Tennessee Criteria) (Sibai 1990). In another set of criteria from the University of Mississippi (Martin et al. 1999), platelets have to be <150,000/μL, SGOT ≥40 U/l, or both, with an increased LDH >600 mg/dL and with evidence of hemolysis (increased LDH and progressive anemia), for a diagnosis of HELLP.

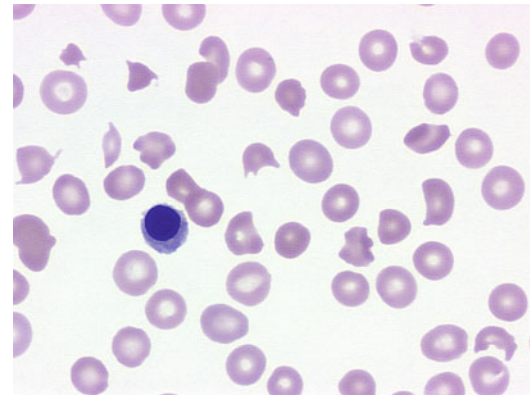

Fig. 17.1 Peripheral smear in a patient with HELLP syndrome, showing microangiopathic changes and low platelet count. Photomicrograph kindly provided by Dr. Karl Theil

Patients usually present with abdominal pain, typically epigastric. It is felt that this is due to progressive enlargement of the liver. In more full-blown preeclampsia or eclampsia, the liver can rupture and one can find evidence of supcapsular hematomas (Suarez et al. 2002). Women may present atypically with shoulder or back pain, upper body aching, headaches, nausea and vomiting. There can often be confusion as to whether a woman has HELLP syndrome, because there is a great deal of overlap with it and DIC, TTP, HUS, acute fatty liver of pregnancy (Ko and Yoshida 2006, and see below), a flare of systemic

lupus erythematosus, or preeclampsia. In these syndromes, other target organs are involved and renal failure may occur. Some women can have two of these disorders occurring simultaneously and treatments can differ for both.

The time course for certain of the elements of HELLP can give the examining obstetrician and hematologist clues as to what diagnosis is occurring. Usually, the platelet count drop occurs first, though if there is preexisting gestational thrombocytopenia, the drop in platelets can merge into the thrombocytopenia of HELLP subtly. LDH usually rises before transaminases. The implication is that hemolysis starts prior to liver injury. In acute fatty liver of pregnancy, transaminases are higher first, elevated bilirubin occurs early and may be more conjugated than unconjugated, PT and PTT rise, serum glucose falls, and then, usually, the platelets begin to fall. TTP differs from acute fatty liver of pregnancy and HELLP because both transaminases are not so prominently elevated in it, especially alanine aminotransferase (SGPT). Usually in HELLP, the magnitude of renal dysfunction mirrors the degree of hepatic dysfunction. IN HUS, however, the renal impairment is more paramount. If the examining physicians are convinced that TTP or HUS are occurring, plasma exchange is started. There is overlap in these syndromes, and it can be very difficult to distinguish them apart.

No one is certain as to the causation of HELLP, however, most theories contend that the placenta is at the root of causation (Zhou et al. 2002). Early on in the HELLP syndrome, activation of the coagulation cascade begins, and there is laying down of fibrin in small vessels (Widurer et al. 2007). The red cells are traumatized as they pass through this fibrin meshwork, leading to microangiopathy and the elevated LDH. Platelets are also consumed within these fibrin clots. Ischemia occurs downstream from these arterioles and capillaries which are full of cross-linked fibrin and the liver parenchyma in the periportal zones demonstrate necrotic changes (Gilbert et al. 2008). Distinguishing HELLP from DIC can be very difficult. If bleeding starts to occur from consumption of clotting factors, surgery, such as emergency C-section, can be very dangerous with massive blood loss.

The best treatment for HELLP syndrome is prompt delivery of the baby and removal of the placenta (Sibai 2007). Magnesium sulfate infusion may help forestall evolution to eclampsia and prevention of seizures or coma (Cahill et al. 2007). If there is a pattern of DIC, with elevated PT and PTT and a drop in fibrinogen, platelet count and red cell numbers, replacement with FFP, cryoprecipitate and platelet and red cell transfusions are necessary. Some clinicians use corticosteroids, though proof of their efficacy is lacking (Amorim et al. 1999). Antihypertensives, especially hydralazine and labetalol are helpful. Sodium nitroprusside is generally avoided because fetal cyanide toxicity has been observed.

Preeclampsia

More is written about causation of preeclampsia than HELLP. In preeclampsia, hypertension and proteinuria predominate (Samuel et al. 2011). Preeclampsia can begin as early as 20 weeks gestation, and can even start as late as 6 weeks postdelivery.

Epidemiology

Preeclampsia and, to a lesser degree, HELLP syndrome tend to occur in women who are pregnant for the first time, in younger mothers, and also older mothers. In women with a history of hypertension, diabetes, or kidney problems, prior to becoming pregnant, incidence of preeclampsia is higher. Also, women with a defined thrombophilia, like FV Leiden, have a greater chance of developing preeclampsia.

Women with a preexisting autoimmune disease, particularly SLE, have a higher risk for preeclampsia and HELLP. Women who have twins (or higher numbers of fetuses) also are at higher risk. The most significant risk factor is having had preeclampsia during a previous pregnancy (Hutcheon et al. 2011).

Preeclampsia Pathophysiology

A theory as to why preeclampsia occurs relates to how the placenta forms in the more superficial

wall of the uterus and becomes hypoxic. There may be an element of maternal immune reactivity against the placenta which leads to this shallow implantation. This leads to immunologically mediated release from the placenta of inflammatory mediators. A subsequent immune response against fetal and paternal antigens ensues. Placental abruption can occur during this process, and is often seen during hypertensive episodes in pregnancy. Evidence for this may be that there is a greater likelihood of finding fetal erythroblasts in the circulation of mothers with preeclampsia compared with mothers who do not develop it. It should be emphasized that tests to predict preeclampsia based on our current knowledge of its pathophysiology are not yet ready for clinical use.

Eclampsia

Eclampsia refers to pregnant women who develop neurologic sequelae to the previously mentioned hypertensive disorders of pregnancy. It is characterized by seizures, usually generalized tonic–clonic, blindness, often reversible, and coma. Eclampsia is not used as a diagnosis for women with a preexisting organic brain disorder. Eclampsia usually evolves from preeclampsia. Magnesium sulfate and diazepam are useful. Hematologically, and from a coagulation perspective, all issues discussed above pertaining to DIC management pertain here, especially FFP, cryoprecipitate, and platelet replacement. It can be very confusing if a woman in the latter stages of pregnancy presents with what appears to be TTP, who has seizures as a manifestation of the neurologic sequelae of TTP. To cover both the possibilities of eclampsia and TTP, evacuating the uterus and plasma exchange may need to be done concurrently.

DIC

DIC is the end result of an insult to the fine balance between clotting and bleeding. Activation of the clotting cascade(s) leads to intravascular thrombus formation with consumption of clotting factors and platelets, which results in bleeding, as well as activation of fibrinolysis. Some individuals with DIC present with bleeding excessively, some clot excessively, and some do both simultaneously. There are several conditions associated with pregnancy that lead to DIC. It is of historical interest that the first reports of DIC occurred in two pregnant women. One had a retained dead fetus and another had abruptio placenta. In the 1901 description of the clotting difficulties of these two patients, the author used the term for DIC as a "temporary hemophilia."

A pregnant woman with DIC is among the most challenging of obstetric presentations. If the fetus is alive, the decision to delay delivery while treating the mothers with transfusions, may threaten its viability. Proceeding with an emergency C-section, at a time when clotting parameters are not optimized, can cause fatal hemorrhage for the mother. Even in circumstances of fetal death, surgery on the mother can lead to fatal bleeding.

Abortion

Without getting into a political discussion of a controversial topic, it is imperative that hematologists are aware of the consultative issues that can arise in a woman who has had an abortion.

The term "septic abortion" refers to infection of the uterus that often occurred in this country before abortion became legal. After Roe vs. Wade was affirmed by the US Supreme Court, providers of abortion practiced in a more medically supervised environment, using sterile instruments, and providing proper postoperative supervision. When a woman has an abortion, infection can occur which can lead to DIC. These women need aggressive treatment with antibiotics and blood/blood product support. Excessive postabortion bleeding can lead to hypotension, consumption of clotting factors and platelets, and so the woman can enter into a DIC-state without having an infection. Even today, death occurs in this setting.

Other Obstetric Conditions Associated with DIC Are Found in Table 17.11

DIC is diagnosed both clinically and in the laboratory. Classically, the PT and PTT are prolonged, the peripheral smear has red cell trauma (schisto-

Table 17.11 Obstetric conditions associated with disseminated intravascular coagulation (DIC)

Septic abortion
Severe HELLP syndrome, or preeclampsia/eclampsia
Amniotic fluid embolism
Placenta previa
Abruptio placenta
Retained dead fetus
Acute fatty liver of pregnancy
Hemorrhage (associated with uterine atony postdelivery, placenta previa, or uterine rupture)

cytosis and polychromasia), fibrinogen is low and fibrin degradation products are elevated. DIC, as a syndrome, can exist along a time-continuum, meaning that one can see a woman early in a DIC state, and the PT might be slightly prolonged, PTT could be normal, platelet count might be normal, and the peripheral smear may be devoid of schistocytes, and the fibrinogen might be normal, or even high. The hematology consultant may confer with the obstetrician and state that right now, at this point, by strict laboratory numbers, the woman might not fit a preestablished criterion for DIC, but clinically, something is happening that is concerning both clinicians. If the woman becomes dyspneic, the BUN and creatinine start to rise, the fetus is showing signs of distress, there appears to be more vaginal bleeding than is normal or there is worrisome oozing from IV sites or the nose or GI tract, and vital signs are changing (hypotension, tachycardia), it is best to recheck DIC parameters quickly, because circumstances can change rapidly with pregnant women. The hematologist should think about the placenta as a separate organ, rich in tissue factor. If that tissue factor starts leaking into the maternal circulation due to any physical or biochemical endothelial damage, one can start along the DIC pathway. If that placenta starts seeing a lower head of pressure from the uterine arteries, its ischemia translates into further tissue factor and other thromboplastin-like substance release, exacerbating the DIC. So, if the first set of DIC numbers seemed reassuring, they should be checked again. If the PT or PTT rise at all, or the platelets or fibrinogen fall any, or the smear seems to be evolving towards RBC fragmentation, the hematologist has to communicate with the obstetrician about the fact that the maternal–fetal unit is heading into trouble and urgent action is needed.

There has been previous discussion in this chapter about DIC that occurs with HELLP, preeclampsia and eclampsia. The reader should refer back to those sections, realizing any of these conditions can rapidly merge into DIC. In acute fatty liver of pregnancy, there is the added feature that once DIC occurs, the liver is not able to compensate and produce more of the clotting factors that are being consumed.

Clinical Vignette 2

You are called by one of your obstetrics colleagues, who with a voice laced with panic asks you to come to the Labor and Delivery Suite immediately. There, you are confronted with a 40-year-old woman, who had three previous normal deliveries, who had an uncomplicated fourth pregnancy, and presented in a very routine way after rupturing her membranes 2 h ago. She was admitted, and had dilated to 4 cm, but suddenly complained of shortness of breath, and rapidly declined, with hypotension, and signs of fetal distress. Labs drawn on admission to the labor unit, which correspond to the time when she started complaining of shortness of breath, demonstrated a white blood count 15,500 with left shift, hemoglobin 9.7 g/dL, platelets 50 k/μL, PT 17 s, PTT 41 s, and fibrinogen 92 mg/dL. You review the smear and there are frequently found schistocytes, polychromasia, and rare nucleated red cells.

Amniotic Fluid Embolism

This catastrophic obstetric condition can occur during any pregnancy, or soon after delivery. It is characterized by the presence of fetal squamous cells in the pulmonary circulation of the mother. At autopsy, the fetal squamous cells can be found in all other organs, though the lungs seem to be the most affected. These squamous cells can be very hard to find. It occurs in 1 in 15,000 deliveries in the USA. In Europe it is 1 in 53,000. Suspected risk factors include rapid tumultuous

labor, trauma, multiparity, increased gestational age, and increased maternal age. Most amniotic fluid embolism (AFE) patients, however, have no clearly identified risk factors (Moore and Baldisseri 2005).

It is imperative to know that maternal death due to AFE can occur within 1 h of symptoms. Anywhere from 40 to 80 % of women with AFE will not survive, depending upon which source one reads. For fetuses still in the uterus at the time of onset, mortality rates are as high as 65 %. For survivors, there is a high risk of neurologic impairment, ranging from mild memory loss to complete anoxic brain injury. Infants may be affected by developmental delay, cerebral palsy, or limited brain function (Knight et al. 2010).

Women affected by AFE present with dyspnea, right heart failure, then left heart failure with hypotension, signs of fetal distress, seizures and DIC. Despite the most optimal resuscitative strategies, which can include recombinant VIIa for bleeding, proper RBC, FFP and platelet replacement for the DIC, ventricular assist devices, inhaled nitric oxide, cardiopulmonary bypass, and intraaortic balloon pump with extracorporeal membrane oxygenation, the outcome for both fetus and mother is usually poor (Gist et al. 2009).

Newer theories of causation suggest an immunologic causation for AFE. One theory suggests the mother suffers an anaphylactic reaction to fetal antigens that become displayed to the maternal immune system during labor. Another theory is that some event causes complement activation. When complement levels are measured in women in the midst of AFE, they are low in most series (Benson 2012).

Clinical Vignette 2 (Continued)

The obstetrician is concerned that the woman is suffering from amniotic fluid embolism, and the DIC numbers confirm his suspicion. He takes her to the operating room for an emergent C-section. The woman bleeds much more than a normal C-section, and is resuscitated with packed red cells, cryoprecipitate, platelets, and fresh frozen plasma. She continues to spiral downhill with intractable hypotension as the baby is delivered with low Apgar scores. In a desperate attempt to save the mother, it is decided to initiate recombinant factor VIIa, at a dose of 90 mcg/kg, and this is repeated every 2 h for the next 10 h. Her lowest platelet count is 10 k/μL. Anesthesiology and the intensivist services initiate inhaled nitric oxide, intraaortic balloon pump, and extracorporeal membrane oxygenation. Over the next 24 h, the bleeding abates. In total, she has been given 40 U of red cells, multiple units of cryoprecipitate, platelets, and plasma. Her DIC numbers get better, and by 36 h after delivery, her platelets are 100,000/μl, PT 12, PTT 36, and fibrinogen 210 mg/dL. The intensive resuscitation measures are withdrawn, and she shows steady and slow improvement. By 1 week after delivery, she is able to be transferred to a regular nursing floor, and with aggressive physical therapy, is able to walk within another week, though her memory and overall strength never recover to the level she was prior to giving birth (Ecker et al. 2012).

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Index

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