22.7.4 Genetic disorders of coagulation 5532 Elean

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section 22 Haematological disorders 5532 Disorders of platelet procoagulant activity Scott syndrome is an extremely rare autosomal recessive condition. Reduced negatively charged phospholipids on the surface of the platelet, normally an important surface for coagulation reactions, results in reduced tenase and prothrombinase activity. FURTHER READING Arnold DM, et al. (2007). Systematic review: efficacy and safety of rituximab for adults with idiopathic thrombocytopenic purpura. Ann Intern Med, 146, 25–33. Bolton-Maggs PH, et al. (2006). A review of inherited platelet disorders with guidelines for their management on behalf of the UKHCDO. Br J Haematol, 135, 603–33. Liang Y, et al. (2012). Rituximab for children with immune thrombo- cytopenia: a systematic review. PLoS One, 7, e36698. Neunert C, et al. (2011). The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. Blood, 117, 4190–207. Provan D, et al. (2010). International consensus report on the inves- tigation and management of primary immune thrombocytopenia. Blood, 115, 168–86. Scully M, et al. (2012). Guidelines in the diagnosis and management of thrombotic thrombocytopenic purpura and other thrombotic microangiopathies. Br J Haematol, 158, 323–35. Shih A, et al. (2014). Novel treatments for immune thrombocytopenia. Presse Med, 43, e87–95. Stasi (2012). How to approach thrombocytopenia. Hematology Am Soc Hematol Educ Program, 2012, 191–7. 22.7.4 Genetic disorders of coagulation Eleanor S. Pollak and Katherine A. High ESSENTIALS Much of what is understood about specific coagulation proteins has emerged from the careful study of hereditary disorders of blood coagulation. Haemophilia Haemophilia is a familial X-linked disorder due to deficiency of ei- ther factor VIII (haemophilia A) or factor IX (haemophilia B), compo- nents of the intrinsic enzymatic complex that activates factor X. The severity of the disease correlates with predicted concentrations of activated factor protein, and those with activity levels below 1% are defined as having severe disease. Clinical features and diagnosis—the main manifestations are bleeding into joints and soft tissues, with haemophilic arthropathy and joint deformity being

inevitable complications in untreated patients. Other features include pseudotumours, bleeding into the urinary system, and bleeding following clinical procedures (e.g. dental extractions). Laboratory diagnosis is based on a modification of the classic activated partial thromboplastin time (APTT) assay, with inhibitor screening used to exclude other causes of prolonged APTT (e.g. lupus anticoagulant). Treatment—Previous methods of administering therapy to Factor VIII and FIX deficient patients continue to advance. Now, rather than providing 'on demand' therapy at specific strenuous times, such as prophylactically before surgery, current treatment seeks to rebal- ance the hemostatic deficiency. The use of recombinant factors is preferable to preparations derived from pooled human plasma sam- ples, which have led to numerous infectious complications (hepatitis
B and C, HIV, and parvovirus. The development of inhibitory anti- bodies is a significant problem, particularly in patients with haemo- philia A. Trials of gene therapy are being performed. Von Willebrand disease Von Willebrand disease is a common autosomal dominant dis- order of platelet function caused by a functional deficiency of von Willebrand factor (VWF). VWF, normally synthesized by mega karyocytes, prevents degradation of factor VIII; VWF, also made by endothelial cells, enhances platelet activation and recruitment at sites of tissue damage. It may be due to quantitative deficiency of VWF (types 1 and 3), or to defect in platelet binding affinity (type 2). Clinical features and diagnosis—typical presentation includes nosebleeds, menorrhagia, and easy bruising. Laboratory diagnosis involves both an antigenic test and an activity test (ristocetin co- factor), in which formalin-fixed platelet aggregation is induced due to ristocetin-enhanced VWF binding to glycoprotein complex Ib–IX. Treatment—mild von Willebrand disease is treated with desmopressin 1-deamino-8-d-arginine vasopressin (DDAVP), which releases factor VIII and VWF from endothelial cells. Other treatments include ε-aminocaproic acid (for patients who require dental sur- gery, and women with menorrhagia), oestrogens, and factor VIII concentrates. Other hereditary disorders of coagulation These include (1) hereditary deficiency of the plasma metalloproteinase ADAMTS13, which predisposes to thrombotic thrombocytopenic purpura; (2) combined deficiency of coagulation factors V and VIII, caused by single-gene defects in the coordinated machinery for pro- tein trafficking and secretion; (3) factor XI deficiency—an autosomal re- cessive diathesis of variable severity frequently occurring in Ashkenazi Jews; (4) inherited deficiencies of factors II, V, VII, and X—these cause bleeding tendencies of varying severity and are inherited as recessive disorders; and (5) deficiency of the contact activating factors, factor XIII, and fibrinogen. Hypercoagulable diseases due to deficiencies of anticoagulants or propensity to thrombosis Typical presentations occur with deep venous thrombosis and/or pulmonary embolism, and hypercoagulable states should be con- sidered particularly with presentation of 'unusual' thrombosis (e.g. superficial thrombophlebitis, mesenteric vein thrombosis, and cere- bral vein thrombosis). Antithrombin III deficiency—diagnosis poses difficulties in the post-thrombotic period when patients frequently have lower levels of antithrombin III due either to consumption of antithrombin III during clot formation or to the decreased function seen with heparin administration. Treatment is typically with therapeutic or prophy- lactic low molecular weight heparin or warfarin; antithrombin III

 concentrate may be given during an acute event or as a prophylactic treatment to prevent further disease. Deficiencies of protein C and protein S—in addition to thrombotic manifestations, protein C deficiency may also manifest as warfarin- induced skin necrosis and dangerously life-threatening purpura fulminans in the homozygous or compound heterozygous protein C-deficient neonate. Factor V Leiden—a single mutation, in the gene

encoding the factor Va protein, leads to prolonged factor Va activity and resist- ance to activated protein C. The factor V Leiden mutation occurs in about 5% of people of European ancestry and may predispose to thrombotic disease. Prothrombin 20210 mutation—the frequent allelic variant (G20210A) in populations of European ancestry increases the con- centration of prothrombin, thus biasing haemostatic balance to- wards excess thrombin formation. Introduction Haemostasis, the physiological process of blood clot formation, involves a coordinated interaction between the wall of the blood vessel, platelets, and blood coagulation proteins. The haemostatic mechanism maintains a state of readiness to respond to a multi- tude of haemostatic stressors to prevent haemorrhage while also preventing inappropriate clot formation. Although acquired dis- eases of the coagulation system frequently occur with liver disease and other pathological disease states, this chapter focuses specific- ally on genetic disorders resulting from abnormalities and/or de- ficiencies of the blood coagulation proteins. More specifically, this chapter covers haemophilia, von Willebrand disease, and deficien- cies/abnormalities of fibrinogen and factors II, V, VII, X, XI, XII, and XIII. The role of an inherited increased risk for excess clotting will also be addressed. These conditions may result from either the loss of function of anticoagulant proteins (antithrombin III, protein C, and protein S) or a gain of function of procoagulant proteins (factor V Leiden and prothrombin 20210G to A). Additionally, we briefly describe haemostasis-related genes: LMAN1 (previously ERGIC-53) and MCFD2 linked to com- bined factor V/factor VIII deficiency, ADAMTS13, associated with thrombotic thrombocytopenic purpura, and the gene for vitamin K epoxide reductase (VKORC1), the enzyme respon- sible for recycling vitamin K 2,3-epoxide to the enzymatically activated form. The coagulation cascade as a haemostatic mechanism The human blood coagulation system involves a coordinated array of reactions that generates a stable fibrin clot when needed and pre- vents unnecessary clot formation. The system involves numerous proteins that interact, principally on phospholipid surfaces, to create a meshwork of fibrin fragments entrapping haematopoietic cells (Fig. 22.7.4.1). The majority of coagulation enzymatic com- plexes involve protease enzymes. Many of these enzymes are serine proteases, and a subset have the distinguishing feature that their functional synthesis requires vitamin K to enable post-translational modification of glutamic acid residues in the N-terminal region; this property provides the basis of the therapeutic mechanism by which the drug warfarin prevents proper synthesis of functional factors. The principal enzyme balancing the pro- and anticoagulant forces is prothrombin, thought to be the evolutionary forerunner of the mammalian coagulation proteins. In addition to its procoagulant functions, prothrombin, once activated, provides anticoagulant and cellular mobility functions as well. In 1905, Morawitz first described the importance of thrombin, thromboplastin, and calcium in cleaving fibrinogen to create a fibrin clot. In the early 1930s and 1940s, laboratory tests were developed that relied on in vitro fibrin clot formation to analyse the adequacy of a patient’s clotting system. The waterfall cascade of sequential ac- tivation steps resulting in a fibrin clot was elegantly described in the early 1960s, delineating separate pathways to account for the pro- thrombin time (PT) and the partial thromboplastin time that the earlier laboratory tests measure. However, the set of activation steps is now better described as an interwoven, reinforcing set of reactions (Fig. 22.7.4.2). The unique specificities of the coagulation enzymes summarized in the classical coagulation cascade have been found to be more versatile in activating diverse proteins under varied condi- tions. However, the separate pathways, now termed the tissue factor (extrinsic) and the intrinsic pathways, help define the steps involved in the principal tests used in clinical medicine for evaluation of haemostatic proteins. For the series of reactions and specific factors involved, the time to clot formation defines the principal parameter used in clinical evaluation of the health of a patient’s coagulation system. The assays (the PT, the

activated partial thromboplastin time (APTT), and activity levels of specific individual clotting factors) Fig. 22.7.4.1 Scanning electron micrograph of a whole blood clot. There is a meshwork of fibrin fibres emanating from platelet aggregates in which erythrocytes, lymphocytes, and other cells are trapped. Courtesy of John W Weisel and Chandrasekaran Nagaswami, Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA.

section 22 Haematological disorders 5534 compare the time needed for clot formation in a patient's plasma with that in a control pool of plasma from normal donors. Endothelial injury and tissue damage first trigger clot formation. The response of the platelets forms the primary phase of healing by temporarily patching the site of vascular injury. Subsequent to this initial platelet phospholipid patch, a fibrin clot provides a more solid framework for the necessary but slower cellular repair. Secondary haemostasis begins with injury-induced exposure of the integral membrane protein tissue factor to plasma proteins, enabling for- mation of the active enzymatic complex tissue factor–factor VIIa. The generation of tissue factor–factor VIIa then catalyses clotting by activating both factor X to factor Xa and factor IX to factor IXa. This activation primarily involves the cleavage of an arginine–isoleucine bond in a secreted plasma protein zymogen to form a two-chain ac- tive protein. Thus, once tissue injury has signalled the need for fibrin clot formation and tissue factor–factor VIIa has initiated coagula- tion, the haemostatic process amplifies through the generation of factor IXa from factor IX, which is 10 times more abundant than factor VII and consequently leads precipitously to thrombin gener- ation. Among thrombin's numerous roles is the activation of the es- sential procoagulant cofactors factors V and VIII. This process then further amplifies clotting by generating more thrombin through the active cofactors Va and VIIIa which then form the tenase (factor IXa/ factor VIIIa) and prothrombinase (factor Xa/factor Va) complexes (Fig. 22.7.4.1). Thrombin also activates the cross-linking enzyme (factor XIIIa) and the fibrinolytic inhibitor (TAFIa), and triggers platelet recruitment. Importantly, thrombin generation simultan-eously counterbalances its procoagulation activities by inciting lysis of the clot via the release by endothelial cells of tissue plasminogen activator converting plasminogen to plasmin, the enzyme respon- sible for fibrin clot lysis. Thrombin also dampens the clotting process by activating protein C that actively breaks down the critical pro- coagulant cofactors factors Va and VIIIa. The basis for initiating clot formation in the PT and APTT tests is titration of calcium into an anticoagulated plasma specimen along with a source of phospholipid. In addition, in the PT test, the source of phospholipid is a thromboplastin reagent that provides tissue factor to enable the tissue factor–factor VIIa complex to catalyse clot formation. The variation in PTs, due to differences in the source of reagent tissue factor, has led to development of the International Sensitivity Index which creates an international normalized ratio (INR) for clinical management and the increasing ability to syn- thesize a thromboplastin with an International Sensitivity Index approaching 1.0. In the APTT test the phospholipid reagent lacks tissue factor and thus prevents formation of the tissue factor–factor VIIa complex. An activator, such as silica particles, also greatly de- creases the time required for clot formation through activation of factor XII via the contact activation system. Deficiencies of specific clotting proteins Haemophilia Deficiency of either factor VIII (haemophilia A) or factor IX (haemophilia B), which together make up the factor VIIIa/factor IXa intrinsic tenase enzymatic complex, results in the clinical pheno- type commonly known as haemophilia. A sex-linked bleeding di- athesis, now thought to be haemophilia, was described in Talmudic writings as a cause of fatal haemorrhage at circumcision. In the modern era, the disease may cause bleeding at circumcision, but haemophilia principally presents with haematoma formation, easy bruising, and bleeding at the site of venepuncture during the tod- dler period. The disease exists in severe,

moderate, and mild forms classified as such on the basis of a clinical laboratory blood coagu- lation test performed to assess the level of functional coagulant protein (percentage activity of factor VIII or factor IX). The patho- logical problem in both haemophilia A (factor VIII deficiency) and haemophilia B (factor IX deficiency; also called Christmas disease), is the inability to form a functional tenase complex to activate factor X to factor Xa. Although factor X can still be activated to factor Xa by tissue factor–factor VIIa, the available quantities of factor VII (400 ng/ml) do not allow sufficient activation of factor X to enable clotting to occur in a physiologically timely fashion. Although pa- tients with haemophilia may have some difficulties with immediate haemorrhage subsequent to a cutaneous or superficial injury, they characteristically have joint and deep tissue bleeding problems as discussed later. The severity of disease is very well predicted by an in vitro assay for evaluation of the deficient protein level such that patients with severe disease have levels of factor activity of less than 1%, patients with moderate disease have activity levels of 1 to 5%, and patients with mild disease have activity levels of 6 to 30%. Normal factor VIII and IX activity levels are 50 to 200% and 75 to 125%, respectively. Numerous genetic mutations have been described accounting for the factor deficiencies causing haemophilia. In part because of the considerable difference in size between the factor VIII gene (186 kb), and the factor IX gene (34 kb), the ratio of the frequency of factor VIII to factor IX deficiency is between 4 and 5 to 1 (c.186/34 kb). PT APTT FXIIa FXI Contact Thr FXIa FIX FVIII FX FIXa FVIIIa Tenase complex Prothrombin Fibrinogen FXIII Fibrin dimers FXIIIa Fibrin monomers Thrombin (Thr) FV Thr FXa FVa Prothrombinase complex TF-FVIIa TISSUE INJURY EXPOSING TISSUE FACTOR (TF) HMWK PL-Ca2+ PL-Ca2+ PL-Ca2+ PL-Ca2+ Thr Thr Fig. 22.7.4.2 Schematic representation of the enzymatic reactions involved in blood clot formation. APTT, activated partial thromboplastin time; F, factor; PL-Ca2+, phospholipids/calcium; PT, prothrombin time; Thr, thrombin.

 Thus, the frequency of haemophilia A is approximately 1 in 5000 to 6000 and that of haemophilia B is approximately one-fifth of that. Among affected cases, approximately one in three to one in four pa- tients presents spontaneously without a familial inheritance pattern. One of the only differences between factor VIII and IX deficiencies is the frequency of severe disease, which occurs more commonly in factor VIII deficiency (60% of cases as compared with 45% in haemophilia B). This difference is largely attributed to the frequency of mutation due to a factor VIII gene inversion in intron 22 of the 26-exon factor VIII gene. At this locus of the factor VIII gene, a re- gion of homology to sequences telomeric to the factor VIII gene, a recombination event results in the inability to synthesize any func- tional factor VIII, thus leading to severe disease (<1% functional protein activity). A less common inversion in intron 1 has also been described in 2 to 3% of severe haemophilia A patients. In both factor VIII and factor IX deficiency, milder disease is commonly due to missense mutations. The clinical features of haemophilia predominantly include bleeding into joints and soft tissues. The incidence of central ner- vous system bleeding has dramatically decreased with concentrate therapy. The average life expectancy of people with severe haemo- philia has increased from 11 years at the beginning of the 20th cen- tury to approximately 70 years at the beginning of the 21st century. However, there was a marked decrease to 60 years in the 1980s, when the devastating effects of blood-borne viral disease again shortened average life expectancy. In the untreated patient with severe disease, haemophilic arthro pathy and joint deformity are inevitable complications. In decreasing order of involvement, the most commonly affected joints include the knee, elbow, ankle, shoulder, wrist, and hip. Recurrent bleeding episodes create a hypertrophic synovial lining with chronic inflam- mation; however, the pathophysiology responsible for recurrent

joint bleeding remains unknown. Arthropathies commonly neces- sitate replacement of affected joints for pain control and improve- ment of mobility. Soft-tissue haemorrhages frequently complicate haemophilia; further complications due to these haemorrhages include compartment syndrome, neurological damage, and exten- sive blood loss from retroperitoneal bleeds. Haematoma formation, a frequent complication of haemophilia, may arise spontaneously or with trauma and require extensive factor replacement and fasciotomy, the necessity for which can be assessed by mean arterial pressure in a compartment. Intracranial haemorrhage, occurring in approximately 5% of patients, warrants immediate evaluation and treatment within the first 6 to 8 h of presentation; however, the majority of children presenting to an emergency department with central nervous system symptoms have not suffered from intracra- nial haemorrhage. A pseudotumour, an encapsulated collection of blood most com- monly originating in bone or soft tissues, is a rare but extremely ser- ious consequence of haemophilia occurring in approximately 2% of patients. This complication is difficult to manage but may sometimes be treated with surgery at specialized haemophilia centres. Frequently, patients with haemophilia have haematuria, the se- verity of which may range from self-limited episodes to gross haema- turia with significant blood loss. Protease inhibitors for HIV therapy may lead to haematuria with flank pain or renal stones. Physicians should be aware of the possibility of nephrotic syndrome in patients with severe disease and high titres of antibody inhibitors receiving high-dose intravenous replacement therapy and other agents to in- duce tolerance. Dental procedures warrant involvement of a haemophilia specialist. Factor replacement levels of 25 to 100% are suggested depending on the complexity of the dental procedure. Antifibrinolytics such as ε- aminocaproic acid (6-aminohexanoic acid) or tranexemic acid and fibrin sealants may be a helpful adjuvant to replacement therapy. On account of the sex-linked inheritance pattern, haemophilia is rarely found in women unless extensive lyonization takes place in the normal factor gene, or the woman is born to a haemophilic father and a carrier mother. Normal vaginal delivery is considered to be relatively safe in the case of a haemophilic infant; however, vacuum extraction, midcavity forceps deliveries, and invasive fetal monitoring should be avoided because of the increased risk of for- mation of subgaleal and cephalic haematomas. The laboratory diagnosis of haemophilia is based on a modifica- tion of the classic APTT assay used as a standard test for the haemo- static system. Normally patients are evaluated due to bleeding symptomatology or because of a prolonged APTT result. The APTT is a very sensitive but poorly specific screening test for haemo- philia. All patients, even those with mild disease, will normally have a prolonged APTT unless there is a problem with specimen acquisition or the insensitivity of the APTT reagent. Once sus- pected, haemophilia can be evaluated by an inhibitor screen which involves performing a 50:50 mix of patient and normal plasma to evaluate whether the prolongation is due to a deficiency of a clot- ting protein or alternatively to the presence of an inhibitor. There are many causes of a prolonged APTT other than haemophilia (see Table 22.7.4.1). Classically, a phospholipid inhibitory antibody, called a lupus anticoagulant, will cause a prolongation of the APTT of the 50:50 mix due to the effect of the phospholipid inhibitory antibody on the normal pooled plasma. A lupus anticoagulant which causes a prolonged APTT may also result in a low factor VIII or factor IX activity level. In such cases, further testing for a lupus anticoagulant is necessary to rule out a low factor VIII due to a lupus anticoagulant as opposed to a deficiency. Previously, management of haemophilia involved administer ing the deficient protein (factor VIII or factor IX) to a patient in a so-called ‘on-demand’ protocol. Subsequent studies established the superiority of prophylactically administered factor concen- trates in terms of clinical outcomes such as joint health, and most patients are now managed of prophylactic regimens. The treatment landscape continues to adjust as new therapy be- comes commercially available. Novel

therapy in the form of a bispecific factor IXa- and factor X-directed antibody, administered subcutaneous weekly or every other week, bypasses the need for intravenous fVIII. This innovative therapy has been licensed for pa- tients (adult and paediatric haaemophilia A) both with or without factor VIII inhibitors. Before the development of stringent purification and viricidal procedures, the transmission of viral disease was almost inevitable as each vial of plasma-derived concentrate was pooled from approxi- mately 60 000 to as many as 400 000 donors, although the number has recently been reduced to 15 000. Tragically, most patients with severe disease treated before 1985 developed HIV. Rates of develop- ment of hepatitis B and C are also extremely high. Although dras- tically reduced, the potential for transmission of infectious disease has not been totally eliminated. Many recombinant preparations are

section 22 Haematological disorders 5536 prepared with human serum albumin, thus leaving a possible source of transfusion of a blood-borne disease. Treatment Acute bleeding episodes Safe and effective treatment options continue to improve for the management of acute bleeding episodes for patients with haemo- philia A and B. Blood products available include fresh frozen plasma which contains both factors VIII and IX, prothrombin complex concentrates containing factors II, VII, IX, and X, activated pro- thrombin complex concentrates (factors IIa, VIIa, IXa, Xa), mono- clonal antibody-purified factor VIII and factor IX, and recombinant factor VIII and factor IX. Recombinant factor VIIa is now approved for use in patients with inhibitors during acute bleeds. Currently, trials using gene therapy approaches are underway and may pro- vide a method for continuous prophylaxis against bleeding (see ‘Future directions: gene transfer as a method of treating haemo- philia’). Recombinant or highly purified products are the optimal therapy because of the great benefit:risk ratio. Availability, ease of administration, cost, viral safety, and thrombotic risk, particularly in patients undergoing high-dose therapy or procedures with a high risk of thrombotic complications, dictate the choice of product. Cryoprecipitate, made from the precipitate of thawed frozen plasma, contains factor VIII but does not contain factor IX. Cryoprecipitate and fresh frozen plasma should only be used in the haemophilia patient in an emergency setting where concentrates are not avail- able. Inhibitor formation, the development of antibodies to the defi- cient protein, arises subsequent to transfusion of a blood product or factor replacement and is the major complication of treatment. An inhibitor presents an extremely difficult situation for patient man- agement (see ‘Complications of therapy’). Several immunoaffinity-purified plasma-derived factor VIII and factor IX products are available in the United States of America and Europe and currently have excellent records of viral safety, effi- cacy, and lack of thrombogenicity. When concentrate is unavailable, fresh frozen plasma is readily available in most emergency settings. Viricidal methods using solvent detergent treatment may now be applied in production of fresh frozen plasma; furthermore, each unit is from a single screened donor, thus the risk of transfusion-transmitted disease is low. Recombinant factor VIII and factor IX have been licensed for over a decade. These proteins are produced in cultured mammalian cells and purified from conditioned medium. Recombinant factor IX is devoid of human plasma whereas the recombinant factor VIII con- centrates utilize human plasma-derived albumin for stabilization. As in vivo coagulant activity of recombinant factor IX is only 80% of in vitro estimates used for labelling of product in international units (IU)/mg, it is recommended that the calculated factor IX dosage be multiplied by a factor of 1.2 for dose calculation when using re- combinant factor IX. A plausible explanation for this discrepancy is a difference in post-translational modifications compared with plasma-derived factor IX. During severe and critical bleeds it is optimal to achieve 50 to 100% factor activity levels for 7 to 10 days (e.g. for pharyngeal, retropharyngeal, retroperitoneal, and central nervous system

bleeds). More modest levels of 20 to 50% for 2 to 7 days are generally adequate for dental extractions, haematuria, intramuscular, or soft- tissue bleeds with dissection, or bleeds into mucous membranes. Levels of 20 to 30% for 1 to 2 days are recommended for uncompli- cated haemarthroses, superficial muscle, or soft-tissue bleeds. The frequency of dosing is every 12 to 24 h for factor IX concentrates and every 8 to 12 h for factor VIII concentrates. At 24 h for factor IX and 12 h for factor VIII, the calculated amount to infuse would be one-half the initial amount of factor IX, as the half-life of factor IX is approximately 18 to 24 h. The timing of factor level determination should be 15 to 30 min after the loading dose and immediately prior to subsequent doses Table 22.7.4.1 Coagulation laboratory testing, plasma concentrations, and chromosomal location of coagulation proteins Deficient clotting factor PT APTT Half-life of protein Plasma concentration Inheritance (chromosome) Fibrinogen Increased Increased 2–4 days 200–400 mg/dl Autosomal (1) Prothrombin (factor II) Increased Increased 3 days 100 μg/ml Autosomal (11) Factor V Increased Increased 36 h 10 μg/ml Autosomal (1) Factor VII Increased Normal 2–6 h 0.5 μg/ml Autosomal (13) Factor VIII Normal Increased 8–12 h 0.1 μg/ml X-linked (X) von Willebrand's factor Normal Mildly increased in approximately 50% of cases Several hours 10 μg/ml Autosomal (12) Factor IX Normal Increased 24 h 5 μg/ml X-linked (X) Factor X Increased Increased 40 h 10 μg/ml Autosomal (13) Factor XI Normal Increased 60–80 h 5 μg/ml Autosomal (4) Factor XII Normal Increased 50 h 30 μg/ml Autosomal (5) Factor XIII Normal Normal 9–19 days 10 μg/ml Autosomal (6: α chain) and (1: β chain) Protein C Normal Normal 6 h 5 μg/ml Autosomal (2) Protein S Normal Normal 30 h 25 μg/ml Autosomal (3) Antithrombin III Normal Normal 48 h 150 μg/ml Autosomal (1) PT, prothrombin time; APTT, activated partial thromboplastin time.

 for appropriate dose adjustments. When factor concentrates are used for patients with inhibitors, higher doses will most likely be re- quired. Additionally, some authors have also reported good success and reduced cost with constant infusion regimens. Calculation of the optimal factor concentration for administration The number of IUs of factor required is equal to: (kg bodyweight) × (desired % factor level increase) × C (kg bodyweight) × (desired % factor level increase) × C where C is a constant depending on the product and the source of the product. The value of C is 0.5 for administration of plasma-purified and recombinant factor VIII, 1 for administration of plasma-purified factor IX, and 1.2 for administration of recombinant factor IX. Surgery in patients with haemophilia When possible, treatment should be instituted by caregivers aware of major and minor adverse reactions and complications occurring in the haemophiliac population. Care should also be given in as- sociation with an experienced reference laboratory able to provide timely evaluation of a patient's response to treatment. Therapeutic factor levels should be obtained before surgery. Depending on the type of surgery, the factor level should reach levels of 50 to 100% of normal and should be maintained 2–7 days post procedure. For brain or prostate surgery, a factor level approaching 100% is re- commended because of the higher risk of bleeding. In patients with milder haemophilia A, administration of the synthetic octapeptide desmopressin (1-deamino-d-arginine vasopressin (DDAVP)) may be helpful in increasing factor levels. However, this is not the case for haemophilia B. In addition to factor concentrates, fibrin glue has been recom- mended with circumcision, antifibrinolytics with dental proced- ures, and recombinant factor VIIa and/or apheresis in patients with high-titre inhibitors. Aprotinin may be considered with caution in cardiac procedures; however, the use of aprotinin has been proposed to increase the risk of thrombogenicity. Complications of therapy The main adverse outcomes related to treatment with concentrates include transmission of viruses when using plasma-derived prod- ucts and development of inhibitory

antibodies seen with both re- combinant and plasma-derived products. Thrombosis has also been a complication of early complex concentrates used for patients with inhibitors. The development of purification schemes that inactivate viruses, and the development of recombinant products, has dramatically de- creased the incidence of transmission of viral disease. Early prepar- ations of prothrombin complex concentrates presented a significant risk of thrombotic complications, but this risk has now been mark- edly reduced. Inhibitor formation, the development of antibodies that in- hibit clotting activity, occurs subsequent to transfusion of a blood product or factor replacement. Development of inhibitors presents difficult challenges for the management of haemophilia. Therapeutic strategies largely rely on the ability to bypass the factor VIIIa–factor IXa tenase complex. Inhibitor formation almost exclusively arises in severely affected patients and occurs in approximately 7 to 52% of patients with haemophilia A but in only about 1 to 3% of patients with haemophilia B. This difference in inhibitor formation is not completely understood, but possible explanations include the higher incidence of severe disease in haemophilia A, prenatal exposure to maternal factor IX but not factor VIII antigens due to the former's ability to pass through the placenta membrane, the structural simi- larity between factor IX and other vitamin K-dependent proteins, the higher plasma levels of factor IX, and the greater inherent im- munogenicity of factor VIII due to its larger size. One very rare but severe complication that may occur with the development of a factor IX inhibitor is the development of a potentially life-threatening ana- phylactic complication following first treatment. Therapeutic strategies for treatment of patients with inhibitors need to address the acute management of the bleeding episode as well as a longer-term treatment directed toward suppression of antibody production. Quantification of the titre of factor inhibitor involves mixing the patient's plasma with test plasma containing a known amount of factor, normally from a pool of healthy donors. After incubation, factor activity levels present in the patient's incu- bation mixture are compared with that in a control mixture so that the amount of inhibitory antibody can be calculated. The Bethesda unit (BU), the standard unit used to report a titre of factor inhibitor, represents the amount of inhibitor that inactivates 50% of factor ac- tivity. Acute management of bleeding in a patient with an inhibitor relies first on quantifying the BU of the inhibitor. With low-titre in- hibitors (<5 BU), it may be possible to overwhelm the inhibitor with aggressive concentrate therapy. With high-titre inhibitors, it is usu- ally necessary to bypass the inhibitor using either prothrombin com- plex concentrates, activated prothrombin complex concentrates, porcine factor VIII, or recombinant factor VIIa. These bypassing agents, with the exception of porcine factor VIII, largely work by dir- ectly activating factor X to factor Xa, thus bypassing the need for the intrinsic tenase complex. With porcine factor VIII, it is wise to first perform testing to ensure that the patient's inhibitor does not cross- react with the porcine factor VIII. Because of the life-threatening bleeding complications in patients with inhibitors, immune toler- ance regimens have been designed with the aim of eradicating the inhibitor in the long term. Therapeutic regimens involve infusion of high-dose factor concentrates enabling a tolerance to the defi- cient factor. This protocol is highly effective in approximately 80% of patients. Viral diseases Severely affected patients treated with plasma-derived concentrates before 1985 had an extremely high rate of viral disease. Over the course of a 70-year lifespan, a patient with severe haemophilia may be exposed to donations from 70 million individuals as a result of the pooling of thousands of donor units for concentrate production. Specific laboratory tests to screen for HIV, hepatitis C, and hepa- titis B, in addition to much improved donor screening procedures, have dramatically limited the number of contaminations from indi- viduals carrying viral diseases. Solvent detergent treatment proced- ures, which inactivate enveloped viruses (HIV and hepatitis viruses B, C, D, and G), and heat treatment

procedures used to eliminate nonenveloped viruses (hepatitis A and E viruses and parvovirus B19) have radically decreased the risk of viral infection from plasma- derived products. The viral inactivation procedures in current

section 22 Haematological disorders 5538 use include pasteurization, vapour heating, high-dry heating, and nanofiltration. Recently, β-propiolactone ultraviolet inactivation has been discontinued because of ineffective viricidal technique. HIV In the late 1970s and early 1980s, HIV appeared in the blood supply before routine laboratory testing was developed to detect its presence. The leading cause of death in American haemophilia patients in 1982 was haemorrhage; however, contaminated blood products during the period between 1979 and 1983 led to a sharp rise in viral disease shortly thereafter. A large proportion of patients with haemophilia became infected with HIV and have subsequently died from AIDS. Risk factors for infection included the severity of the disease (severely affected patients were much more commonly affected than those with moderate or mild disease), the type of concentrate used (factor VIII vs factor IX concentrate), the viral inactivation procedures used in product preparation, and the geo- graphical location of the patient with regard to percentage of blood products contaminated. The incidence of HIV infection in American patients who received plasma-derived concentrates between 1979 and 1984 was lower in patients receiving factor IX complex concentrates (55%) than in those receiving factor VIII concentrates (approximately 90%). Despite the devastating consequences of HIV for affected individ- uals and families, the projected impact on births of patients with haemophilia over the next two centuries is small (1.79% reduction). Hepatitides Contaminated plasma-derived products also led to significant morbidity and mortality due to hepatitis viruses. Effective viricidal techniques have greatly reduced the incidence of hepatitic viral disease in this population. In the United States of America in the late 1980s, 87% of the 345 HIV-negative and more than 99% of the HIV-positive patients showed evidence of prior infection with hepatitis B, hepatitis C, or hepatitis D viruses. Infection due to hepa- titis A virus has rarely been reported in patients with haemophilia in the United States of America. Solvent/detergent inactivation of con- centrates has been associated with a high prevalence of antibodies to hepatitis A virus. Hepatitis B was commonly seen in patients with haemophilia until routine screening of liver enzymes and the subsequent availability of hepatitis-specific antibody and antigen tests in the 1980s. Most patients are now vaccinated against hepatitis B so that it is difficult to estimate hepatitis B infection from concentrate administration. The hepatitis delta virus, dependent on coinfection with hepatitis B virus, has also been a significant cause of illness in patients with haemophilia; its prevalence is largely attributed to the administra- tion of prothrombin complex concentrates. Routine testing for hepatitis C, instituted in the early 1990s, has reduced but not eliminated hepatitis C contamination in the donor pool. A variable susceptibility and morbidity is seen in response to hepatitis infection; cirrhosis was estimated at approximately 20% and liver failure at 10 to 20% 20 years after infection. Concurrent infection with HIV can accelerate complications of hepatitis C virus. There is also an increased likelihood of hepatocellular carcinoma with long-term infection with viral hepatitis. Other infectious agents The great majority of patients with haemo- philia have antibodies to parvovirus B19. This is a small, nonlipid- enveloped, highly heat-resistant virus found to contaminate plasma-derived products. Methods that inactivate other nonenveloped viruses in products have not proved to be routinely effective against this virus. Although parvovirus B19 infection is often mild and self-limited, infection with parvovirus B19 has the potential to severely compromise the health of an infected immunodeficient patient. There is experimental evidence in animal models that cellular blood components, plasma, and plasma components have a po- tential, though minimal, risk of

transmitting the prion disease Creutzfeldt–Jakob disease (CJD). To date, no definitive direct infection of a recipient of a blood product or blood product concentrate has been documented, although transmission has been reported from corneal and dura mater grafts and cadaveric pituitary hormones. The American Red Cross currently administers a question- naire to screen donors for risk of prion disease. There is now concern that transmission of new variant Creutzfeldt–Jakob disease (vCJD) may differ from classical CJD and could potentially be transmitted through plasma-derived concentrates. This new variant has been associated with outbreaks of bovine spongiform encephalopathy, potentially from dietary exposure. Experimental evidence shows that bovine spongiform encephalopathy and vCJD are caused by the same infectious agent, and prion-related protein has been found in lymphoid tissue of patients with vCJD. In Europe, large amounts of plasma products have been removed from the market as a result of concerns about the risk of vCJD. Treatment of patients infected with hepatitis virus
and/or HIV Vaccination against hepatitis A and B is highly recommended for patients who receive concentrates and lack viral antibodies indi- cative of past infection. Treatment of hepatitis C with direct acting antivirals has excellent rates (>80%) of sustained virological re- sponse. Liver transplantation has been successful in many cases for patients with liver failure who are unresponsive to treatment. The liver transplant fortuitously corrects the deficiency of clotting protein due to synthesis of clotting factors by the orthotopic liver. However, the possibility of reinfection with viral disease is signifi- cant and must be included in management decisions. Therapies continue to rapidly improve for HIV treatment. Healthcare providers for patients with both haemophilia and HIV must keep abreast of changing HIV therapies and work alongside infectious disease specialists. Drug-related hepatitis in haemophilia patients has been reported subsequent to treatment of HIV, particu- larly in response to indinavir. Additionally, complications in HIV- positive haemophilia patients taking protease inhibitors include haematuria, intracranial bleeds, and excessive bleeding often re- quiring hospitalization and administration of higher than expected doses of factor concentrate to correct the bleeding. Protease inhibitor therapy should not be withheld from HIV-positive individuals with haemophilia. A 6-month prospective study of 20 haemophilia pa- tients receiving protease inhibitors revealed only one unusual bleed, which was corrected by factor infusion. Future directions: gene transfer as a method of treating haemophilia The development of clotting factor concentrates resulted in a dramatic improvement in life expectancy for individuals with haemophilia. Nonetheless, this treatment strategy has a number of disadvantages. The protein must be infused intravenously, and has

 a relatively short half-life in the circulation. This makes chronic prophylaxis difficult, especially in small children where venous ac- cess may present a problem. In addition, the product is so expensive that only about one-quarter of the world’s patients with haemophilia (those in the developed world) have access to it. Although current viral inactivation techniques have largely eliminated the risk of HIV and hepatitis in plasma-derived products, there are ongoing con- cerns about the risk of other blood-borne diseases (CJD, transfusion- transmitted viruses) that are not easily eradicated using current techniques. These concerns have fuelled interest in the development of a gene transfer approach to the treatment of haemophilia. Such an approach, if successful, would result in continuous production of a level of clotting factor adequate to prevent bleeds rather than treating bleeds after they have occurred. The level of clotting factor required for this goal can be predicted based on a generation of experience with clotting factor concentrates. Thus, in Swedish prophy- laxis studies, it has been shown that maintenance of trough factor levels in the range of 1 to 3% are adequate to prevent all

the life- threatening bleeds and most of the joint bleeds in boys with severe haemophilia. Data from a large natural history study show that pa- tients with mild haemophilia born with circulating FVIII levels 12% have annualized bleeding rates of zero. These data provide bench- mark goals for levels of expression in gene therapy. Successful gene transfer approaches require three elements: a therapeutic transgene, a means of delivering it (i.e. a vector), and an appropriate target cell type in which gene transfer and expression will exert a therapeutic effect. Of the inherited diseases for which gene transfer approaches have been attempted, haemophilia has a number of advantages. First, tissue-specific expression is not required. Although clotting factors are normally synthesized in the liver, bio- logically active material can be synthesized in a variety of tissues, including fibroblasts, muscle cells, and endothelial cells. This allows latitude in the choice of target cell. Second, the therapeutic window is wide, since even small increases in circulating levels of factor are likely to result in some improvement in symptoms, and increases up to 100% would provide the patient with physiological normal activity levels. Excellent small and large animal models of the diseases exist (murine and canine), and determination of therapeutic efficacy is in the case of haemophilia relatively straightforward, since levels of circulating factor correlate well with symptoms of the disease. A number of different strategies for gene therapy for haemophilia have been investigated in preclinical studies, and five of these were evaluated in early phase clinical trials conducted in the United States of America beginning in 1999. The plethora of approaches suggests that there may eventually be more than one successful combination of vector and target tissue that is safe and effective for haemophilia; this is an advantage, since the haemophilia population is a hetero- geneous one, and approaches that work well in some instances (e.g. delivery of a viral vector to the liver), may be impossible for indi- viduals with severe liver disease due to prior hepatitis C infection. One of these original studies, in which an AAV vector expressing human factor IX was infused into the hepatic artery in men with severe haemophilia B, resulted in expression of therapeutic levels of factor IX, in the range of 10 to 12%, for a period of several weeks, but expression was gradually lost. The decline in factor IX levels was ac- companied by an asymptomatic and self-limited rise in serum trans- aminases. Studies of the immune response to vector in a subsequent subject documented an expansion of a population of capsid-specific CD8+ T cells following vector infusion; this population contracted after the loss of factor IX expression. In vitro studies demonstrated that peptides derived from the AAV vector are displayed on the sur- face of the transduced cell in the context of MHC class I molecules; the molecular basis of the loss of expression and rise in liver trans- aminases then appeared to be presentation of AAV-derived capsid sequences on the surface of the transduced hepatocyte, which flagged them for destruction by circulating lymphocytes. Because the only source of capsid is the material that is infused, that is, there is not ongoing synthesis of capsid proteins, the conclusion was that a short course of immunomodulatory therapy, administered in re- sponse to a rise in liver enzymes or a fall in factor IX levels, would block the response and allow long-term expression. In a subsequent trial that used an AAV8 vector with a self-complementary DNA structure and a codon-optimized sequence, provision was made to institute a short course of high-dose prednisolone if indicated. This proved effective and participants infused at the highest dose, 2×1012 vg/kg, have shown long-term expression (at last report, up to 8 years with observation ongoing) of circulating factor IX levels in the range of 4 to 6%, enough to convert severe haemophilia B to mild disease. A key advance in this trial was the realization that the strong tropism of AAV vectors for the liver permitted intravenous infusion of the vector rather than infusion through the hepatic artery in an interventional radiology procedure. Subsequent trials have substi- tuted a high specific activity Factor IX molecule (Factor IX Padua) for native Factor IX; this has driven durable expression of

Factor IX in the range of 30%, at lower doses of AAV vector (5 × 1011 vg/kg). The large size of the factor VIII cDNA slowed efforts to extend this approach to haemophilia A, but multiple trials of AAV-mediated gene transfer, some in late phase testing, are now underway. The re- sults in the AAV studies underscore the iterative nature of advances in gene transfer, with problems encountered in early phase testing in humans requiring additional studies in the laboratory or in preclin- ical animal models to inform the next generation of clinical studies. Von Willebrand's disease In 1926, Erik von Willebrand first described what we now know as von Willebrand's disease upon finding an autosomally in- herited bleeding diathesis in a large kindred on the Åland islands in the Gulf of Bothnia between Sweden and Finland. Although the bleeding disorder in this family resulted in haemorrhagic death in multiple family members, the bleeding diathesis in pa- tients with von Willebrand's disease is usually much milder. Most commonly, patients with von Willebrand's disease manifest mu- cosal platelet-type bleeding tendencies of varying severity. Nose bleeds, menorrhagia, and easy bruising are the most common manifestations. The pathophysiology of von Willebrand's disease involves a functional deficiency of von Willebrand factor (VWF), a 270-kDa monomer that forms a large multimeric plasma glycoprotein com- prised of several up to 100 subunits. Synthesized in the megakaryocyte and endothelial cell and stored in subcellular granules, VWF enables proper two-chain factor VIII formation and serves as a carrier, thus preventing degradation of factor VIII and lengthening the half-life of the labile factor VIII protein to around 8 h. VWF secreted by endothelial cells also binds to heparin glycosaminoglycan and to the platelet glycoprotein complex Ib–IX enhancing platelet activa- tion and further platelet recruitment at sites of tissue damage. The

section 22 Haematological disorders 5540 interaction between platelets and VWF is thought to provide the explanation for the mucosal bleeding phenotype occurring in pa- tients with von Willebrand's disease. These patients frequently have reduced levels of factor VIII. However, the remaining factor VIII is normally sufficient to prevent the haemophilia-type symptom- atology of arthropathy and deep tissue bleeding. The 180-kb gene for VWF is located on chromosome 12 and consists of 52 exons. There are three types of von Willebrand's dis- ease: types 1, 2, and 3. Types 1 and 3 are quantitative deficiencies of the VWF, but type 2 is a qualitative deficiency due to binding defects of the VWF. The inheritance of types 1 and 3 are autosomal dom- inant and autosomal recessive, respectively. However, rare reports of an autosomal dominant inheritance pattern for type 3 have been published. There are four principal subtypes of type 2 classified as follows: 2A, absence of high molecular weight VWF species causing decreased platelet-dependent function; 2B, increased affinity of VWF for platelet glycoprotein Ib–IX; 2M, platelet functional defect not caused by the absence of high molecular weight multimers; and 2N, a factor VIII binding abnormality (Table 22.7.4.2). Laboratory diagnosis of von Willebrand's disease involves as- saying the plasma for VWF. The two principal tests are an antigenic test (VWF antigen) and an activity test (VWF ristocetin cofactor) in which formalin-fixed platelet aggregation is induced due to the ristocetin-enhanced VWF binding to glycoprotein complex Ib–IX. Comparison of the tests helps identify the enhanced ristocetin- induced aggregation seen in type 2B von Willebrand's disease where VWF ristocetin cofactor is typically much lower than VWF antigen. Other tests performed in the evaluation of von Willebrand's disease include the level of factor VIII, which is often decreased, and the APTT, which is elevated in approximately half of cases of von Willebrand's disease due to the low activity of factor VIII. Nonreducing gel immunoelectrophoresis is employed to assay the distribution of multimeric subunits of VWF with a gel containing antibody to VWF antigen. This assay is particularly relevant for visu- alization of the presence of low, intermediate, and high

molecular weight VWF subunits. The intermediate and high molecular weight species are markedly decreased in subtypes of type 2 disease. A de- creased normal pattern is seen in type 1 disease, although the de- creased visual intensity may be difficult to quantify. Type 3 disease shows near absence of all subunit molecular weights. The lower limit of the normal range of VWF varies with blood type (A, B, O, AB). Thus, symptomatology must be evaluated based on normal ranges for each blood type. The bleeding time in a patient with von Willebrand's disease is most often prolonged; however, the test is no longer routinely necessary because of the nonspecific nature of a positive result and the higher specificity of other testing. The specific treatment for von Willebrand's disease varies with a patient's symptoms, the circumstances of the need for treatment, the subtype of von Willebrand's disease, laboratory results indicating the potential success of increased VWF with nonprotein-based treat- ment, and the clinical experience with a particular patient and the biological family members. When possible, treatment is preferred without blood products. The mainstay of treatment for mild disease is treatment with the synthetic octapeptide desmopressin, DDAVP. This causes release of factor VIII and VWF from endothelial cells raising the plasma VWF by approximately two- to tenfold. Thus treatment with DDAVP relies on a partial quantitative deficiency of VWF. Intravenous and nasal preparations are available. The nasal preparation allows a patient to self-administer medication at ei- ther regular intervals or on an as-needed basis. The phenomenon of tachyphylaxis, the decreased effectiveness of repeated doses of the compound, does occur, and there is usually little response after three consecutive doses. In the past, DDAVP was considered contraindi- cated in type 2B von Willebrand's disease because of the thrombo- cytopenia sometimes observed with DDAVP infusion. However, this recommendation is controversial and should be assessed on a case-by-case basis. Patients with type 3 von Willebrand's disease may lack sufficient intracellular reserves for effective therapy; thus alternative meas- ures for such patients are usually necessary. A better understanding of VWF function in vivo exposes limitations with current clinical testing for VWD activity measured during static conditions. These testing difficulties largely result from a lack of simulated physiologic flow environments. Using functional flow-based tests along with collagen and platelets may help better analyse patient disease ac- tivity. New practical tests may help safely classify patients as higher or lower risk for clinical bleeding. A trial of effectiveness of DDAVP is often indicated, particu- larly prior to prophylactic surgical use of the compound. The trial is normally performed after subtyping the VWF disease to ensure that DDAVP is not contraindicated, as in type 2B. Optimally, the test should not be given within 24 h of the last DDAVP infusion nor at a time of environmental stress in order to minimize problems as- sociated with tachyphylaxis or depletion of intracellular reserves.

A therapeutic trial entails measurement of VWF antigen before and 1 h after DDAVP infusion of 0.3 μg/kg. The patient should be moni- tored carefully during this period because of possible flushing, mild anaphylactoid reactions, and possible hyponatraemia. ε-Aminocaproic acid is frequently administered in the setting of dental surgery to inhibit fibrinolysis. However, care must be taken in administration to patients with a predisposition to thrombosis because of the potential deleterious effects of ε-aminocaproic acid in this setting. Other compounds which may be administered include oestrogens in women because of the natural positive regulation of synthesis of VWF with oestrogen compounds. This may ameliorate menorrhagia in such patients. Components in cryoprecipitate in- clude factor VIII, fibrinogen, and factor XIII, in addition to VWF. Cryoprecipitate had been the mainstay of plasma-based therapy until the recent availability of factor VIII concentrates with pre- served VWF protein such as Alphanate and Humate P. The use of cryoprecipitate, which does not undergo viral inactivation, has thus fallen out of favour. Treatment of von Willebrand's disease with DDAVP is the method of choice in patients who respond to this therapy. DDAVP for intra- venous or

subcutaneous use is supplied as either a 4-µg/ml 10-ml vial or a 15-µg/ml 1- or 2-ml vial preparation. The recommended Table 22.7.4.2 Subtypes of von Willebrand disease type 2 Subtype Change in binding affinity Characteristics 2A Decreased platelet binding Loss of high molecular weight multimers 2B Increased platelet GP1α binding Thrombocytopenia 2N Decreased factor VIII binding Low factor VIII levels 2M Decreased factor VIII binding Normal multimers

22.7.4 Genetic disorders of coagulation 5541 dose is 0.3 µg/kg, mixed in 30 ml normal saline, infused slowly over 30 min or 0.4 µg/kg subcutaneously. This dose may be repeated after 12 to 24 h. A DDAVP nasal spray is available in a metered dose pump which delivers 0.1 ml (150 µg) per actuation. The bottle is at a con- centration of 1.5 mg/ml and contains 2.5 ml with a nasal spray pump which can deliver twenty-five 150-µg or twelve 300-µg doses. For administration, patients who weigh less than 50 kg should deliver one 150-µg spray in one nostril. For those weighing over 50 kg, one spray should be delivered in each nostril for a total dose of 300 µg. Administration may be repeated after 24 h. Precautions to take with the medication include administration no more than every 24 h or for three consecutive days unless under the supervision of personnel from a haemophilia treatment centre. The medication should not be used in pregnant women or in children under 2 years of age. The medication should be used with caution in the elderly and in indi- viduals with a history of cardiovascular disease. ADAMTS13 deficiency—thrombotic thrombocytopenic purpura The gene encoding the novel metalloproteinase ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type- 1 motifs) was discovered in 2001. ADAMTS13, located on chromo- some 9q34, cleaves the peptide bond at Tyr1605 to Met1606 in the A2 domain of VWF. Normally, ADAMTS13 rapidly degrades ‘unusually large’ VWF multimers into smaller multimers. Lack of ADAMTS13 due to familial absence or acquired inhibition may result in thrombotic thrombocytopenic purpura with an increase of the ultra-large multimers and formation of platelet clumps and microthrombi (see also Chapter 22.7.4). Combined deficiency of coagulation cofactors factor V and factor VIII Combined deficiency of coagulation cofactors factor V and factor VIII (F5F8D) is a rare autosomal recessive disorder due to genetic mutations in the coordinated system of protein trafficking. The dis- order results from mutations in the genes for the transmembrane lectin LMAN-1 (ERGIC-53) on chromosome 18 (18q21.3–q22) or its protein complex partner MCFD2 (multiple coagulation factor deficiency 2) located on chromosome 2 (2p21). MCFD2 recruits glycoproteins factors V and VIII for endoplasmic reticulum–Golgi transport by the molecular chaperone ERGIC-53. LMAN1 or MCFD2 mutations that cause this rare disorder in patients result in indistinguishable clinical manifestations with mild to moderate bleeding symptomatology. Plasma antigen and activity levels of both factors V and VIII measure between 50 and 300 U/L. Factor XI deficiency Factor XI deficiency is an autosomal recessive bleeding diathesis of variable severity. It was first described in 1953 as a third type of haemophilia and is thus sometimes referred to as haemophilia C or Rosenthal syndrome. The deficiency predominantly occurs in eastern European Ashkenazi Jews, accounting for more than 50% of cases. In Ashkenazi Jews, the disorder is reported to occur in 5 to 11% of individuals in the heterozygous state and 0.1 to 0.3% in the homozygous state. Genetically, the mutations are grouped into three types: type I, abnormalities in the intron–exon splice boundaries; type II, mutations that result in a premature stop in translation; and type III, mutations resulting from a missense mutation. The protein itself is an 80-kDa protein that circulates in the plasma as a zymogen in a noncovalent association with high molecular weight kininogen. It contains four apple domains in its protein structure, and although factor XIa is a cleaving protease, its structure differs from the vitamin K-dependent serine protease coagulation proteins. Factor XI is principally activated by factor XIIa in the pres- ence of a negatively charged

surface (contact activation). The lack of any bleeding diathesis related to a severe deficiency of factor XII suggests the importance of thrombin as an alternative mechanism of in vivo factor XI activation. The in vitro factor XI activity level does not correlate well with clinical phenotype. Family history of the bleeding complications and the specific mutated sites are more predictive. Bleeding manifest- ations are rare in heterozygotes and occur in approximately 50% of homozygous patients. Factor XI activity levels are assayed in an APTT-based test. Bleeding problems include easy bruising, epistaxis, haematuria, post- partum haemorrhage, haematomas, and menorrhagia. Haemophilia symptoms, including haemarthroses and intramuscular bleeding, are rare. Bleeding most frequently occurs after trauma or surgery. Damage to tissues rich in fibrinolytic activity, such as oral mucosa and the prostate, are more commonly associated with bleeding problems. Therapy for patients with factor XI deficiency is indicated for symptomatic bleeding and prophylactically for surgery in pa- tients with markedly reduced levels (i.e. <20%), unless there is no personal or family history of any bleeding complication. Fresh frozen plasma should be readily available at surgery for infusion in case of a bleeding emergency. Factor XI has a half-life of 60 to 80 h; 10 ml plasma/kg per day is usually adequate for maintaining haemostasis. Prophylactic therapy for most surgery includes re- placement of factor XI with plasma at a loading dose of 15 ml/kg followed by 3 to 6 ml/kg every 24 h. The protective level for surgical prophylaxis is suggested as 45% for major surgery and 30% for minor surgery. Antifibrinolytic therapy with ε-aminocaproic may be a helpful adjunct to plasma therapy; however, antifibrinolytics should be avoided in patients with haematuria or bleeding in the bladder be- cause of possible obstruction by clots. Deficiencies of proteins in the tissue factor and common pathways The autosomally inherited deficiencies of factors II, V, VII, and X re- sult in bleeding diatheses of varying severity. Such deficiencies of co- agulation factor correlate poorly with tests of in vitro factor activity; these are thus quite different disorders from haemophilia, in which in vitro assessment predicts the clinical phenotype very well. These factor deficiencies can best be assessed by an initial screen using the PT as a measurement of the tissue factor pathway. Although the APTT may be prolonged with deficiencies of factors II, V, and X, but not VII, the PT is most often much more sensitive. Factors II, VII, and X, are structurally homologous containing a signal peptide, a propeptide region necessary for recognition by the post-translational modifying enzyme γ-glutamyl carboxylase, an intermolecular binding region (two epidermal growth factor (EGF) domains in factors VII, IX, and X and two kringle domains in the prothrombin molecule), and a catalytic domain in the C-terminal of the molecule.

section 22 Haematological disorders 5542 Deficiency of prothrombin (factor II) results from a lack of pro- thrombin or a malfunctional prothrombin protein. Deficient patients present with haemorrhagic manifestations. All reported patients with a prothrombin deficiency retain some prothrombin, suggesting that complete prothrombin deficiency is incompatible with life. This is consistent with the knockout mouse model which results in em- bryonic lethality at 9.5 to 11.5 days postcoitum in over 50% of fetuses; however, for some unknown reason, some murine knockout pro- thrombin fetuses are able to survive to birth but promptly die within 2 days due to haemorrhage. Patients with heterozygous prothrombin deficiency most commonly are either asymptomatic or have min- imal bleeding. Bleeding manifestations include easy bruising, soft-tissue haemorrhage, excessive postoperative bleeding, epistaxis, and menorrhagia in women. Haemarthroses are uncommon. Congenital disease is characterized by a lifelong and a family bleeding history. Levels of 20 to 30% prothrombin normally prevent symptomatic bleeding. When necessary, administration of plasma is recommended at doses of 15 to 20 ml/kg followed by 3 ml/kg every 12 to 24 h. Prothrombin complex concentrates can be administered for serious bleeds

and as prophylaxis before surgery. Transmission of viral disease and thromboembolic phenomena are risks of the ad- ministration of prothrombin complex concentrates. Factor V deficiency occurs in fewer than one in a million indi- viduals. Approximately 20% of the body's factor V reserve resides in the platelets. Thus, it is not surprising that patients with factor V deficiency tend to have mucosal bleeding manifestations including epistaxis, gastrointestinal bleeds, and menorrhagia in women. Haemarthroses, although a possible complaint, are much less common than in haemophilia. Mild to moderate bleeding may be treated by raising the factor V activity to about 20% of normal with a plasma dose of approximately 15 to 20 ml/kg followed by 3 to 6 ml/ kg every 24 h. Due to the large amount of factor V stored in α gran- ules, platelet transfusions may be an appropriate therapy. However, patients should be monitored for the possibility of generation of antiplatelet antibodies. Factor VII deficiency presents as a variable bleeding disorder ranging from mild to severe, with a possibility of fatal intracranial haemorrhage. Patients with homozygous or compound hetero- zygous mutations manifest symptoms similar to those of a patient with haemophilia. However, unlike the correlation between activity levels and severity of disease in haemophilia, the in vitro factor VII activity clotting test provides only a relative indication of possible disease manifestations. This, in part, is caused by different tissue factor origins utilized within clinical laboratory thromboplastins. Manifestations of factor VII deficiency include haemarthrosis, arthropathies, haematoma formation, and retroperitoneal bleeding. Fatal intracranial haemorrhage is estimated to occur in approxi- mately 16% of patients with severe disease. Levels below 10% ac- tivity most often result in bleeding manifestations. Therapy includes replacement of factor VII activity levels to 10 to 25% for patients undergoing most types of surgery. Treatment options include plasma at 5 to 10 ml/kg for 6 to 12 h for 1 to 2 days for minor episodes. For surgery, the recommended dose is administration of 15 to 20 ml/kg followed by maintenance doses of 3 to 6 ml/kg every 12 h. Prothrombin complex concentrates may frequently be used to supply the factor VII along with the other vitamin K-dependent proteins. Although thrombogenicity has only been reported on rare occasions, this does remain a minor yet potential complication. In July 2005, recombinant coagulation factor VIIa (NovoSeven) was officially approved in the United States of America for treating pa- tients with factor VII deficiency. Intravenous doses of 15 to 30 μg/kg are therapeutically effective in this setting for acute bleeds, a signifi- cantly lower dose than that used for treatment of haemophilia pa- tients with inhibitors. However, the possible development of a factor VII inhibitor must be considered, as this has been reported. The product is administered every 2 h for prophylaxis during surgery for the first 24 h, then reduced to every 3 h 24 to 48 h postoperatively, and then further reduced according to patient symptomatology and necessity, depending on the risk of bleeding into the surgical site. Factor X deficiency may present with symptomatology similar to that of a patient with severe haemophilia. Haemarthroses, soft tissue haemorrhages, retroperitoneal bleed, central nervous system haem- orrhages, pseudotumours, and menorrhagia may occur. Therapy with fresh frozen plasma includes a loading dose of 10 to 15 ml/kg followed by approximately 50% of that at 24 h. Deficiency of the contact activating factors, factor XIII, and fibrinogen Although the APTT is grossly prolonged (often >150 s) with de- ficiencies of the contact activating factors—factor XII, high mo- lecular weight kininogen, and prekallikrein—these deficiencies are not associated with bleeding manifestations and will not be covered further here. Factor XIII deficiency often presents shortly after birth with bleeding of the umbilical cord. Patients with clinical manifest- ations typically have factor levels of less than 1%. Factor XIII is a transglutaminase that cross-links fibrin monomers, thus stabilizing a forming fibrin clot. Patients with deficiency of factor XIII therefore have delayed wound healing and often suffer from soft tissue haem- orrhages, haemarthroses, haematomas, and excessive bleeding from poorly

healed wounds. Up to 25% of individuals deficient in factor XIII may experience intracranial bleeding. For unknown reasons, affected men may have oligospermia and affected women may suffer from repeated spontaneous abortions. Since routine clotting tests are normal in factor XIII deficiency, a physician must specifically request a test for factor XIII deficiency which entails a clot solubility test using 2% chloroacetic acid on a formed clot. Treatment of factor XIII deficiency involves administration of small amounts of factor XIII required to minimize bleeding complications. Prophylaxis in- cludes using 2 to 3 ml/kg of fresh frozen plasma every 4 to 6 weeks or one bag of cryoprecipitate per 10 to 20 kg every 3 to 4 weeks. To prevent spontaneous abortions, products containing factor XIII can be administered every 14 to 21 days. Afibrinogenaemia may cause dangerous haemorrhagic episodes. However, it is somewhat surprising that the mutation does not lead to embryonic death in light of the fact that the blood is incoagulable in vitro. The lack of necessity for fibrinogen during fetal develop- ment is supported by the viable fibrinogen knockout mouse model. Prolonged bleeding from the umbilical cord often permits early recognition of an affected child. The leading cause of death in afibrinogenaemia is intracranial haemorrhage. Haemorrhages from mucous membranes occur frequently, and haemarthroses occur in approximately 20% of patients. Pregnancy-related problems in- clude first-trimester abortion, placental abruption, and postpartum bleeding complications and may be markedly reduced by adminis- tration of fibrinogen. However, fibrinogen replacement may cause

22.7.4 Genetic disorders of coagulation 5543 thromboembolic phenomena. The target fibrinogen level for re- placement therapy is approximately 50 to 100 mg/dl. One bag of cryoprecipitate contains approximately 250 mg of fibrinogen; thus dosing of cryoprecipitate usually necessitates 5 to 10 bags per 70 kg person. Therapeutic complications include allergic reactions and the development of antifibrinogen antibodies. Thromboembolic phenomena may occur in conjunction with fibrinolytic inhibitors or oral contraceptives. Dysfibrinogenaemia results from a functional deficiency of fi- brinogen associated with a malfunctioning molecule, although some degree of antigen remains present. Approximately 55% of pa- tients with dysfibrinogenaemia remain asymptomatic, 25% have a bleeding tendency, and 20% may experience thrombotic episodes ranging from mild to fatal events. Combined defects Numerous combined deficiencies have been described; the under- lying mutation for several of these combined deficiencies has been determined. Combined deficiency of the two structurally similar proteins factor V and factor VIII is an autosomal recessively inherited disorder of variable bleeding severity (mentioned previously). Other combined deficiencies for which a genetic mechanism has been de- scribed include deficiency of factors II, VII, IX, and X caused by a mutation in the γ-glutamyl carboxylase gene, required for a critical post-translational modification in vitamin K-dependent factors. Vitamin K clotting deficiency subtypes 1 and 2 result from func- tional deficiencies of enzymes γ-glutamyl carboxylase and VKORC, respectively. Symptoms occur not only from defective functions of all vitamin K-dependent coagulant factors (both pro and inhibitory) but defective protein γ-carboxylation results in nonhaemostatic de- velopmental and skeletal abnormalities. Hypercoagulable disease due to deficiencies of anticoagulant Pathological diseases resulting from inappropriate clot formation in either the arterial or venous circulation is a major cause of mor- bidity and mortality worldwide. The genetic contribution to this pathophysiology is not well understood, particularly concerning thrombosis in the arterial circulation. Clearly cardiovascular disease represents a complex multifactorial process. The contribution of genetic factors to venous thrombotic disease is better understood; it may be associated with either an isolated deficiency of an anticoagulant protein, a malfunctioning procoagu- lant protein, or a combination of these

processes. The functional deficiencies become particularly relevant during times of increased environmental stress such as in the puerperium or in postsurgical, traumatic, or immobilized states. In addition to deficiency states, several common mutations involving a gain of function have also been described which can disrupt the delicate balance of coagula- tion by shifting the balance toward greater procoagulant function. Procoagulant and anticoagulant plasma proteins interact with platelets and cellular phospholipids to promote physiological co- agulation. Regulation of thrombin formation is the key step in the proper balance between pro- and anticoagulant functions. Anticoagulant proteins are particularly important in areas where there may be prolonged exposure of procoagulant factors and platelet phospholipids to the vessel wall, predisposing an individual to thrombotic disease. Deficiencies of anticoagulant proteins thus place a patient at an increased risk for thrombosis in the slowly flowing venous circulation. In the rapidly flowing arterial circula- tion, laminar flow largely prevents prolonged interaction between platelets and vessel walls. The principal anticoagulant proteins that keep the procoagulant proteins in check include thrombomodulin, tissue factor pathway in- hibitor, antithrombin III, protein C, and protein S. Thrombomodulin, an integral membrane protein expressed by endothelial cells, plays a key role in tempering the action of thrombin. Despite attempts to discover mutations in the thrombomodulin gene, only rare reports have impli- cated thrombomodulin in the pathophysiology of disease, although some recent studies suggest the existence of polymorphic regulation variants in the promoter region. Recently, a mutation in the small but critical protein known as tissue factor pathway inhibitor, which inhibits procoagulant function by binding to factor Xa either alone or in asso- ciation with tissue factor–factor VIIa, has been suggested to be associ- ated with a ninefold increased risk of venous thrombosis. Deficiencies leading to a hypercoagulable state are most fre- quently caused by deficiencies of antithrombin III, protein C, and protein S. These anticoagulant deficiencies result from either a quantitative deficiency (type I) or a qualitative deficiency (type II). Deficiencies of any of these factors may cause life-threatening deep venous thromboses and pulmonary emboli, or may be asymptom- atic. Clinical presentation relates to physical sequelae in the affected organ. In addition to deep venous thromboses and pulmonary em- boli, symptomatology may include superficial thrombophlebitis, mesenteric vein thrombosis, and cerebral vein thrombosis. Antithrombin III deficiency A deficiency of antithrombin III was the first anticoagulant pro- tein deficiency described to be associated with an increased risk of thrombosis. Antithrombin III is a 60-kDa glycoprotein found at high concentrations in the plasma—150 µg/ml: approximately 15- to 30- fold higher than that of many other pro- and anticoagulant proteins. Antithrombin III primarily inhibits thrombin but also inhibits fac- tors IXa, Xa, XIa, XIIa, kallikrein, and plasmin. The ability to inhibit thrombin requires interaction with heparin, which increases the in- hibitory activity several thousandfold. Historically, the risk of throm- bosis in individuals deficient in antithrombin III has been thought to be higher than that seen with deficiencies of protein S or protein C, or than that seen with increased functionality of the procoagulant pro- teins factor V and prothrombin. Clearly the influences of gene–gene and gene–environment interactions contribute to this risk. A normal activity range for most procoagulant/anticoagulant proteins may be as low as 50%. However, the critical requirement for antithrombin III can be surmised from the 80% lower limit of a normal antithrombin III level, significantly higher than that for other coagulation proteins. This makes the diagnosis of antithrombin III deficiency particularly difficult in the post-thrombotic period when patients frequently have lower levels of antithrombin III due either to consumption of antithrombin III during clot formation or to the decreased func- tion seen with heparin administration. Additionally, the presence of homozygous disease of antithrombin III deficiency has only been re- ported with rare

type II deficiencies resulting from impaired heparin binding mutations. No homozygous type I deficiencies have been re- ported, probably because of their incompatibility with life.

section 22 Haematological disorders 5544 The frequency of antithrombin III deficiency in patients with thrombophilia varies widely between studies. The cause of these differing frequencies has recently been carefully addressed by van Boven and colleagues. Their study clearly shows the strong influ- ence of acquired and genetic factors which modulate the baseline risk due to one specific genetic mutation, highlighting the role of additional factors when combined with genetics. In thrombophilic family studies, the risk of thrombosis is 20 times greater than in con- trol populations. The most frequent presentation is deep venous thrombosis with a pulmonary embolism, particularly after an inciting environmental influence such as surgery or immobiliza- tion, or the start of oral contraceptives or pregnancy/postpartum in women. The average age of first onset is 33 years. In patients deficient in antithrombin III without a known acquired risk, the rate of inci- dence of thrombosis is less than 1% per year. Therapy for antithrombin III deficiency includes prophylactic treatment with warfarin, low molecular weight heparin, and treat- ment of an acute event with heparin or another anticoagulant therapy, for example administration of a fibrinolytic agent in the patient pre- senting early enough during an acute episode. Antithrombin III concentrate may be administered for therapy of deficiency during an acute event or as a prophylactic treatment to prevent further disease. Deficiencies of proteins C and S Deficiencies of proteins C and S present with thrombotic mani- festations similar to those seen with antithrombin III deficiency. However, in protein C deficiency an additional complication includes warfarin-induced skin necrosis and life-threatening pur- pura fulminans in the homozygous or compound heterozygous protein C deficient neonate. A diagnosis of protein C deficiency is found in approximately 33% of individuals with warfarin-induced skin necrosis, a development that may lead to skin necrosis several days after initiation of warfarin therapy. The proposed mechanism for this condition is due to the earlier decrease in protein C com- pared with decreases in procoagulant proteins following initiation of warfarin therapy (due to the short half-life of protein C, c.6 h). It is thus standard clinical practice to begin warfarin only after a patient has first been anticoagulated with heparin or another immediately- acting anticoagulant therapy. Protein C acts in concert with its cofactor protein S to inacti- vate the active forms of the procoagulant cofactors, factors Va and VIIIa. Protein C is a vitamin K-dependent serine protease structur- ally similar to factors VII, IX, and X. Protein S is also vitamin K dependent because of the conserved N-terminus but lacks enzym- atic function because of the existence of a sex-hormone binding globulin domain instead of a catalytic domain at the C-terminus. Thrombin activates protein C to activated protein C when bound to thrombomodulin, a protein that acts like an endothelial cell receptor for thrombin. Symptomatic manifestations of protein C or protein S deficiencies are similar to those of antithrombin III deficiency. Deep venous thrombosis with or without pulmonary embolism occurs in 50% of patients by the age of 30 to 45 years, depending on the study population. Environmental and gene–gene interactions are par- ticularly important. As with antithrombin III deficiency, superficial thrombophlebitis, cerebral vein thrombosis, and mesenteric vein thrombosis are all possible complications. Postphlebitic syndrome presents as a complication after deep venous thrombosis in up to 50% of patients. Deficiencies of protein Z Like coagulant protein C and protein S, protein Z serves as a vitamin K-dependent anticoagulant protein regulated by membrane surface- associated procoagulant proteins. The 62- kDa vitamin K-dependent single-chain glycoprotein Z acts as a cofactor to the enzyme protein Z-dependent protease inhibitor (ZPI), a catalytically active serpin. Clinical, animal, and meta-analytic studies provide evidence that ZPI and/or PZ deficiency can be

associated with thrombotic complications. The risk of problems amplifies with compounding haemostatic stresses including pregnancy, and concurrent arterial or venous abnormalities. Hypercoagulable mutations Factor V Leiden and prothrombin 20210 mutation Since 1994, two additional common mutations have been described leading to an increased risk of thrombosis. These mutations, unlike the anticoagulant protein deficiencies, are due to gain-of-function mutations causing either an increased resistance to inactivation in factor V (factor V Leiden) or increased levels of a procoagulant protein (pro- thrombin) which results in higher levels of thrombin formation. Activated protein C (APC) resistance was first described by Dahlback in a 42-year-old man with a history of recurrent throm- boses. Dahlback noted an absence of prolongation of the APTT found after addition of APC, which is normally prolonged due to inactivation of factors Va and VIIIa. Soon thereafter, Poort and col- leagues identified a single mutation as the principal cause of APC resistance in the vast majority (over 90%) of patients. The mutation leads to a decreased ability of APC to inactivate the cofactor Va due to an amino acid substitution (arginine for glutamine) at a critical hydrolysis point in the factor Va protein normally enabling inacti- vation. Other causes of APC resistance not due to factor V Leiden include a haplotype in the factor V molecule, the H2 haplotype. Factor V Leiden leads to thrombotic disease as described for hypercoagulable states due to deficiencies of anticoagulant protein. Due to the extremely high incidence of factor V Leiden in the white population (c.5%), gene–gene interactions play a particularly im- portant role in manifestation of disease. It should be noted that the frequency of factor V Leiden in most nonwhite populations is low. The prothrombin 20210 mutation reported in 1996 results in an increased concentration of prothrombin, also tipping the balance towards excess thrombin formation. The cause of this increase is as- sociated with a guanine to adenine mutation (G20210A) at the last base of the 3′ untranslated region in the factor V gene. The mech- anism by which this influences prothrombin levels is thought to be post-transcriptional. Factor IX Padua The occurrence of a nonhaemophilia mutation at amino acid 338 had been predicted in 1993 due to its location at a cytosine–guanine (CpG) hotspot. In Padua in 2009, such an X-linked factor IX mutation was identified resulting in an arginine (R) to leucine (L) (factor IX Padua) substitution. The (R338L) substitution does not cause factor IX deficiency but conversely correlates with a gain-of-function mutation resulting in a 5- to 10-fold elevated factor IX coagulant ac- tivity. The hyperfunctional (R338L) substitution in animal experi- mentation reveals the potential to utilize this mutation in gene-based future designs to treat haemophilia B patients with inhibitors.

22.7.4 Genetic disorders of coagulation 5545 Vitamin K epoxide reductase complex, subunit 1 The gene encoding vitamin K epoxide reductase (VKOR), the en- zyme that completes the vitamin K cycle, was identified in 2004. VKOR converts vitamin K 2,3-epoxide to the enzymatically activated form. The gene was independently identified by two distinct tech- niques, a traditional positional cloning approach and a novel small interfering RNA-aided functional screen of the predicted locus on chromosome 16. VKOR is a multisubunit complex; VKOR complex, subunit 1 (VKORC1) is a 163-amino acid integral membrane protein (18 kDa) highly expressed in the liver. The oral anticoagulant war- farin, used in the prophylaxis of acute and chronic thromboembolic conditions, inhibits the action of vitamin K-dependent proteins. Pharmacogenetics-based dosing algorithms based on VKORC1 genotyping have been developed to account for interindividual variation in patient response to warfarin. Additionally, differences in warfarin metabolism, largely based on variant cytochrome P450 complex alleles, are used when initiating warfarin dosing. In addition, a free website (http://www.warfarindosing.org) has been created to help pharmacogenetically guide and adjust warfarin doses for patients and their prescribing physicians. Direct-acting oral anticoagulants Better understanding of dangers and drawbacks of warfarin have paralleled a

timely appearance of new direct-acting oral anticoagu- lants (DOACs). In general, these new drugs have more predictable pharmacogenetic responses, fewer interactions with food and other medications, and display much wider therapeutic windows. Compared to warfarin, DOACs display shorter half-lives and achieve their full therapeutic effect in hours as opposed to days. Variations exist between available DOACs (dabigatran, apixaban, rivaroxaban, and edoxaban) regarding creatinine clearance, me- tabolism by cytochrome P450 isoforms, and protein binding. Additionally, DOACs require little to no regular monitoring, under known conditions. Therapy using the new drugs has become a recommended treatment prophylactic option for both short term and long-term thrombophrophylaxis for prevention and management of atrial fib- rillation, stroke as well as venous thrombosis. The prior major con- cern of the lack of reversibility has been resolved with availability of new agents that counteract several DOACs. Time of last dosage remains vital in knowing if, when, and how to manage excess drug. Treatment of treat life-threatening and uncontrolled bleeds has been established with Idarucimab to counteract dabigatran as well as andexanet alpha to overcome an excess of apixaban and rivaroxaban. Issues still remain regarding higher medication cost compared with warfarin therapy, in addition to a small but increased risk of bleeding with some agents. However, the decrease in time and expense due to previous laboratory monitoring in addition to the availability of re- versal agent protocols has helped outweigh most drawbacks. FURTHER READING General articles about coagulation Colman RW, et al. (2006). Overview of hemostasis. In: Colman RW, et al. (eds) Thrombosis and haemorrhage, 5th edition, pp. 3–16. JB Lippincott, Philadelphia. Davie EW, Ratnoff OD (1964). Waterfall sequence for intrinsic blood clotting. Science, 145, 1310–12. Gage BF, Lesko LJ (2008). Pharmacogenetics of warfarin: regulatory, scientific, and clinical issues. J Thromb Thrombolysis, 25, 45–51. Li T, et al. (2004). Identification of the gene for vitamin K epoxide re- ductase. Nature, 427, 493–4. Levy GG, et al. (2001). Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. Nature, 413, 488–94. MacFarlane RG (1964). An enzyme cascade in the blood clotting mech- anism and its function as a biochemical amplifier. Nature, 202, 498–9. Nichols WC, et al. (1998). Mutations in the ER-Golgi intermediate compartment protein ERGIC-53 cause combined deficiency of co- agulation factors V and VIII. Cell, 93, 61–70. Roberts HR, Lozier JN (1992). New perspectives on the coagulation cascade. Hosp Pract, 27, 97–105, 109–12. Zhang B, et al. (2008). Genotype-phenotype correlation in combined deficiency of factor V and factor VIII. Blood, 111, 5592–600. Haemophilia and von Willebrand’s disease Berntorp E, et al. (2006). Inhibitor treatment in haemophilias A and B: summary statement for the 2006 international consensus confer- ence. Haemophilia, 12 Suppl 6, 1–7. Bolton-Maggs PH (2006). Optimal haemophilia care versus the reality. Br J Haematol, 132, 671–82. Bolton-Maggs PH, et al. (2004). Evidence-based treatment of haemo- philia. Haemophilia, 10 Suppl 4, 20–4. Ljung R, et al. (2019). Inhibitors in haemophilia A and B: Management of bleeds, inhibitor eradication and strategies for difficult-to-treat patients. Eur J Haematol, 102(2), 111–22. Ota S, et al. (2007). Definitions for haemophilia prophylaxis and its outcomes: the Canadian consensus study. Haemophilia, 13, 12–20. Plug I, et al. (2006). Mortality and causes of death in patients with hemophilia, 1992–2001: a prospective cohort study. J Thromb Haemost, 4, 510–6. Ragni MV (2006). The hemophilias: factor VIII and factor IX deficien- cies. In: Young N, Gerson S, High K (eds) Clinical hematology, pp. 814–29. Elsevier, Philadelphia. Sadler JE, et al. (2006). Update on the pathophysiology and classifica- tion of von Willebrand disease: a report of the Subcommittee on von Willebrand Factor. J Thromb Haemost, 4, 2103–14. Administration of factor concentrates Ewenstein BM, et al. (2004). Consensus recommendations for use of cen- tral venous access devices in haemophilia. Haemophilia, 10, 629–48. Federici AB, Mannucci PM (2007). Management of inherited von Willebrand disease in 2007. Ann Med, 39, 346–58. Manco-Johnson MJ, et al. (2007). Prophylaxis versus episodic treat-

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