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An Approach to a Patient with Bleeding Disorder

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ABSTRACT

Hemorrhagic diathesis can be caused by disorders in primary hemostasis (von Willebrand disease, inherited platelet function disorders), secondary hemostasis (hemophilia A, B, other coagulant factor deficiencies) and fibrinolysis, and in connective tissue or vascular formation. This review summarizes the approach to a bleeding patient starting from structured patient history, to applying bleeding assessment tools (BATs), the utilization of currently available diagnostic methods for bleeding disorders and finally investigating with highly specialized tests. A comprehensive framework for a genetic diagnostic work up to inherited bleeding disorders is the need of the hour. The discovery of a wide spectrum of mutations in the various genes associated with coagulation and their association with different severity of the disease has allowed for the development of a rational strategy for mutation detection in clinical settings. Characterization of the genetic defects is also required for carrier detection, antenatal testing, and predicting risk of factor inhibitor development.

KEYWORDS

bleeding disorder, approach, investigations, genetics

INTRODUCTION

Hemostasis involves interactions between the blood vessels, platelets and coagulation factors[1]. A defect in any of these phases of coagulation can result in a bleeding problem which may be inherited or acquired. The aim of hemostasis is to prevent blood loss following injury by the formation of a stable blood clot. Blood clots are eventually dissolved by the fibrinolytic system. A delicate balance between formation and dissolution of a blood clot is maintained[2,3]. A disruption of this unique balance results in bleeding or thrombosis. The objectives of this review is to provide primary care physicians with a systematic diagnostic approach in dealing with patients suffering from bleeding disorders, and demonstrate the importance of clinical and laboratory evaluation as highlighted in Table 1.

Table 1: Evaluation of a patient with bleeding disorder

Clinical evaluation	History Family history Bleeding assessment tools (BATs) Physical examination
Laboratory evaluation	First line investigations (screening tests) Second line investigations (confirmatory test) Third line investigations (genetic workup)

History taking: A good detailed comprehensive history is the best predictor of a bleeding problem.

NATURE OF BLEEDING

If the bleeding involves the skin (cutaneous) and mucous membranes i.e. petechiae, purpura bruises, epistaxis, gingival bleeding, menorrhagia and/or hematuria, it would suggest a platelet and/or vascular abnormality. Bleeding into deep tissue, joints and muscles suggest a coagulation factor defect[4].

HISTORY SUGGESTING CONGENITAL/ACQUIRED BLEEDING

A history of easy bruising or exaggerated bleeding after injury suggests an inherited bleeding problem. There should be questions on any childhood history of epistaxis, umbilical stump bleeding, bleeding after circumcision hinting an inherited bleeding disorder. Any history of blood transfusion or other blood components is very important. Information on all surgeries including tooth extractions and any history of abnormal bleeding during or after surgery should be evaluated. The response to trauma is an excellent screening test. Any significant injury without abnormal bleeding is good evidence against the presence of an inherited haemorrhagic disorder.

A very careful family history is critical; any family history of abnormal bleeding in parents, maternal grandparents, aunts, uncles, and siblings as well as any history of consanguineous marriage (or among relatives) should be taken. However, a negative family history does not exclude an inherited coagulation disorder. As an example, up to 30-40% of patients with hemophilia A have a negative family history. A comprehensive history will guide the direction and extent of the laboratory evaluation and also help in determining management.

MEDICATION USE

A careful history of medication use is important, including prescribed medications, over-the-counter medications and herbal products. Drugs cause bleeding due to thrombocytopenia or platelet dysfunction, aplastic anemia or vascular purpura.

Bleeding scores (BSs) have been proposed for obtaining standardized quantitative histories[5]. Because symptoms might be subtle, especially in younger children, a detailed clinical history is crucial. In order to standardize this, questionnaires have been drafted and are known as bleeding assessment tools (BATs). The main advantages of using BATs are:

1. As a screening tool, particularly for mild bleeding disorders.

2. To assess disease severity.
3. To address phenotypic heterogeneity and correlations between clinical and laboratory phenotype, and between genotype and phenotype
4. To improve communication in a clinical setting.

PHYSICAL EXAMINATION

From the clinical assessment, one should be able to assess whether: (1) the bleeding is the result of a local anatomic defect or part of a systemic defect in hemostasis, (2) the bleeding is due to a vascular defect, platelet abnormality or coagulation disorder, or (3) the haemostatic defect is inherited or acquired.

DISORDERS OF PLATELETS OR BLOOD VESSELS

They are characteristically associated with mucosal (epistaxis, gingival) and cutaneous bleeding (petechiae, ecchymoses). Patients with platelet abnormalities tend to bleed immediately after vascular trauma and rarely experience delayed bleeding, which is more common in the coagulation disorders.

COAGULATION DISORDERS

The typical manifestations of bleeding in the coagulation disorders are large palpable ecchymoses and large spreading deep soft tissue hematomas. Haemorrhage into synovial joints most often indicates a severe inherited coagulation disorder such as hemophilia.

LABORATORY INVESTIGATIONS

For each phase of hemostasis, screening tests which help in distinguishing a platelet disorder from a coagulation defect are available. The use of these tests are never a substitute for clinical assessment, as there is enough evidence that screening tests are unhelpful in the prediction of a bleeding disorder especially when applied indiscriminately. The quality of the report issued from the laboratory will depend on the quality of the sample. Clotted, hemolyzed and inappropriately collected sample will lead to erroneous results. Sodium citrate is the anti-coagulant of choice.

First line investigations (screening tests) include a complete blood count (CBC) to assess platelet count, peripheral blood smear examination, prothrombin time (PT), activated partial thromboplastin time (APTT), a thrombin time (TT) and a bleeding time (BT) or platelet function analysis (PFA)[6,7,8]. Second line investigations include mixing studies, factor levels, platelet aggregation studies and von Willebrand factor level.

Platelet counting and the peripheral smear — Platelets may be counted directly or with the use of fully automated electronic methods. While some automated methods may flag for the presence of unusually small or extremely large platelets, there is no substitute for direct examination of the peripheral blood smear for detection of quantitative as well as qualitative (ie, abnormalities of platelet size) platelet abnormalities. Examination of the peripheral blood smear is essential in patients with low platelet counts to exclude the presence of pseudothrombocytopenia due to in vitro platelet agglutination in the presence of EDTA. This phenomenon is thought to result from a "naturally occurring" platelet autoantibody directed against a normally concealed epitope on the platelet membrane, which becomes exposed by EDTA. Use of alternative anticoagulants (eg, citrate or heparin), may circumvent this technical problem.

Bleeding time — The bleeding time (BT) is a measure of the interaction of platelets with the blood vessel wall. A prolonged bleeding time may occur in thrombocytopenia (platelet count usually below 50,000/microL), qualitative platelet abnormalities (eg, uremia), von Willebrand disease (VWD), some cases of vascular purpura, and severe fibrinogen deficiency, in which it is probably the result of platelet dysfunction. Among patients with a normal platelet count who are not taking aspirin, the bleeding time is used primarily to screen patients for inherited disorders of platelet function. An abnormal test in a patient with mucocutaneous bleeding would justify further testing for platelet dysfunction or specific tests for von Willebrand disease (VWD). However, a normal value for the BT should not preclude testing for VWD. Platelet Function Analyzer is more sensitive for detection of VWD than is the BT.

A normal BT does not predict the safety of surgical procedures, nor does an abnormal BT predict for excessive bleeding. Since assessment of the BT is subject to considerable variation due to technical factors in executing the test, a normal range for the test varies from laboratory to laboratory, and cannot be generalized here. Of importance, the BT is not recommended as a preoperative screening test. Because of considerable variation due to technical factors in executing the test, the BT plays a limited role, if any, in evaluating hemostatic defects.

The Platelet Function Analyzer — The commercially-available Platelet Function Analyzer (PFA-100) is an alternative technology that assesses platelet function with greater sensitivity and reproducibility than the bleeding time (BT). Because the BT is insensitive, invasive, time consuming, and subject to variation due to technical factors, many centers have adopted the PFA-100 in place of the BT as their screening test of platelet function. This test may be performed on citrated samples of whole blood that have been stored at room temperature, and is considerably faster to perform than platelet aggregation studies. Normal PFA-100 test results may obviate the need for further expensive platelet function testing. Unlike the in vivo BT, the PFA-100 test does not provide a measure of vascular function.

Prothrombin time — The production of fibrin via the extrinsic pathway and the final common pathway (common to both extrinsic and intrinsic cascades) requires tissue thromboplastin (tissue factor), factor VII (extrinsic pathway), and factors X, V, prothrombin (factor II), and fibrinogen. The functioning of these pathways is measured by the plasma prothrombin time. The test bypasses the intrinsic pathway and uses thromboplastins to substitute for platelets. Within this combined pathway, factors VII, X, and prothrombin are vitamin-K dependent and are altered by warfarin. For this reason, the PT is used

Table 2: Screening coagulogram for a patient with bleeding disorder

Test	Normal range
Platelet count	150-450 x 10^3/cmm
Platelet morphology	Size and clumps
Clot retraction study	Good
Prothrombin time	10-14 seconds
Activated partial thromboplastin time	25-38 seconds
Thrombin time	15-19 seconds
Clot solubility test	Insoluble

as a measure of the anticoagulant activity of warfarin and other vitamin K antagonists.

Activated partial thromboplastin time — The activated partial thromboplastin time (aPTT) measures the intrinsic and common pathways of coagulation. It is called partial since platelet substitutes are used which are only partial thromboplastins; they are incapable of activating the extrinsic pathway, which requires complete tissue thromboplastin (tissue factor). In the original method, a glass test tube provided contact activation. However, the addition of activators such as ellagic acid or particulate silicates provided better and more standardized contact activation. This activated version of the PTT (aPTT) is now the routine assay used to evaluate intrinsic coagulation and the degree of heparin anticoagulation.

The aPTT is sensitive to inhibitors such as heparin and to deficiencies of all coagulation factors except factors VII and XIII. It is less sensitive than the PT to deficiencies of the common pathway (factors X and V, prothrombin, and fibrinogen). High levels of a single factor (eg, factor VIII) can shorten the aPTT. However, an association between a short aPTT and a hypercoagulable state remains controversial.

Thrombin time and reptilase time — The thrombin time (TT) and reptilase time (RT) measure conversion of fibrinogen to fibrin monomers and the formation of initial clot by thrombin and reptilase, respectively. Reptilase, a thrombin-like snake enzyme, differs from thrombin by generating fibrinopeptide A but not fibrinopeptide B from fibrinogen and by resisting inhibition by heparin via antithrombin. Fibrin strand cross-linking, which is mediated by factor XIII, is not measured by these assays.

Prolonged thrombin times and reptilase times may be due to hypofibrinogenemia, structurally abnormal fibrinogens (dysfibrinogens), or increased fibrin split products. Since heparin prolongs the TT but not the RT, the RT is useful for determining if heparin is the cause of a prolonged TT. Alternatively, one can test for heparin activity via its anti-factor Xa activity, or with the use of a commercial heparinase.

Factor deficiencies and inhibitors — A prolonged aPTT can be due to a deficiency (or absence) of a coagulation factor or the presence of a coagulation factor inhibitor. A factor deficiency should be correctable by addition of normal plasma to the test reaction tube. This is normally done by performing a PT or aPTT on a 1:1 mixture of patient and normal plasma (mixing study). Specific factor deficiencies are then determined by assessing the PT or aPTT in mixes of test plasma with commercially available plasmas deficient in known factors. Factor levels can be functionally assessed by comparing test results to standard curves generated by mixtures of serially diluted normal plasma and factor-deficient plasma. Immunologic assays can also be used to measure factor levels. Immunologic and functional assays should give equivalent results when a factor deficiency is present. On the other hand, a low functional assay but normal immunologic assay indicates the presence of a functionally abnormal factor.

The presence of a factor inhibitor is suspected when the abnormal test does not correct, or only partially corrects, following an immediate assay of a 1:1 mixture of patient and normal plasma. In some cases, such as acquired factor VIII antibodies, the aPTT may correct immediately after mixing, but becomes prolonged after 60 to 120 minutes of incubation at 37º. In addition to factor inhibitors, lupus anticoagulants can result in a prolonged aPTT that is not correctable by the addition of normal plasma. The effect of these antibodies on the aPTT can be overcome by adding excess platelet phospholipid (particularly a hexagonal phase phospholipid) or by assessing the diluted Russell's viper venom time. Paradoxically, the antiphospholipid syndrome is usually associated with a tendency to thrombosis rather than bleeding; the prolonged aPTT is an artifact of the antiphospholipid phenomenon.

Tests for fibrinolysis — Fibrin and fibrinogen degradation products (FDP) are protein fragments resulting from the action of plasmin on fibrin or fibrinogen, respectively. Elevated levels are seen in states of fibrinolysis such as disseminated intravascular coagulation (DIC). FDP assays do not differentiate between fibrin degradation products and fibrinogen degradation products. It is possible to accurately measure the concentration of fibrin D-dimers, which are degradation products of cross-linked fibrin. The method of choice is the enzyme-linked immunosorbent assay (ELISA).

When fibrinolysis exceeds thrombin generation, thereby increasing the risk of hemorrhage rather than thrombosis (eg, disseminated intravascular coagulation associated with acute promyelocytic leukemia), quantitative FDP levels may be more sensitive than D-dimer levels as an indication of the degree of fibrinolytic activity. Because D-dimers specifically reflect fibrinolysis of cross-linked fibrin (ie, the fibrin clot), assessment of D-dimer levels suggests thrombosis more reliably. As an example, in patients who have a low pretest probability of deep vein thrombosis, the negative predictive value of D-dimers is high.

The euglobulin lysis time, which assesses overall fibrinolysis is less useful, since results from this test may vary significantly in relation to calcium ion concentrations as well as plasma levels of tissue plasminogen activator and plasminogen activator inhibitor-1. Alpha-2 antiplasmin, an inhibitor of fibrinolysis, is not measured in this test.

Table 3: Screening test results in various bleeding disorders

Platelet count	Prothrombin time	Activated partial thromboplastin time	Differential diagnosis
Normal	Increased	Normal	Factor VII deficiency(early liver disease, early vitamin K deficiency, early warfarin therapy) Dysfibrinogenemia Factor VII inhibitor Some cases of DIC
Normal	Normal	Increased	Factor VIII, IX, XI deficiency Inhibitors to Factor VIII, IX, XI VWD Heparin Lupus inhibitor
Normal	Increased	Increased	Vitamin K deficiency Liver disease Warfarin Heparin Factor V,X, deficiency
Decreased	Increased	Increased	DIC Liver disease HIT
Decreased	Normal	Normal	Increased platelet destruction Decreased platelet production Hypersplenism
Increased	Normal	Normal	Myeloproliferative disorders
Normal	Normal	Normal	Mild VWD Qualitative platelet defects Factor XIII deficiency

More specific tests of the fibrinolytic system include assays for plasminogen, tissue plasminogen activator (t-PA), alpha-2 antiplasmin, plasminogen activator inhibitor-1 (PAI-1), and thrombin-activatable fibrinolysis inhibitor (TAFI). Assays for alpha-2 antiplasmin are used clinically to identify patients with alpha-2 antiplasmin deficiency, an inherited disorder associated with delayed bleeding. However, specific assays for t-PA, PAI-1 and TAFI are of uncertain use clinically.

The screening coagulogram is tabulated in Table 2.

The aim of the screening tests is thus, to reveal broadly the source of problem, and accordingly request further investigations. Interpretation of screening tests is tabulated in Table 3.

The second line investigations include mixing studies in the case of abnormal PT or aPTT for further information on the nature of the defect; coagulation factor assays to confirm and assess the severity of the coagulation factor deficiency such as in Hemophilia A or B; platelet aggregation studies to confirm platelet qualitative defects and investigations for Von Willebrand disease. If all baseline-screening tests are normal then investigations for factor XIII deficiency and alpha 2-antiplasmin deficiency which are not detectable by the routine screening tests are warranted. Factor XIII deficiency may be diagnosed by a clot solubility test, and the alpha 2-antiplasmin activity can be measured by a chromogenic assay. If all investigations are found normal, the patient should be investigated for blood vessel wall abnormalities. A vessel wall defect can result in abnormal bleeding despite an otherwise normal coagulation system. Since there are no reliable clinical tests of vascular integrity, diagnosis depends on a high level of suspicion, when all laboratory tests are normal.

BLEEDING DISORDERS WITH NORMAL SCREENING COAGULOGRAM

There can be scenarios in which the coagulation disorders leading to a bleeding diathesis may be associated with normal screening coagulation tests (i) if the factor is not involved in the steps in coagulation measured by in vitro tests or (ii) if the degree of deficiency is mild. The most common amongst these rare clinical disorders is factor XIII deficiency[9]. Factor XIII stabilizes and cross-links fibrin strands. Factor XIII deficiency may present with delayed bleeding, usually 24 to 36 hours after surgery or trauma. Coagulation testing shows normal values for the PT, aPTT and TT. The diagnosis is made by measurement of reduced plasma factor XIII activity; an immunoassay for factor XIII or demonstration of clot dissolution in 5 molar urea or monochloroacetic acid. Rare abnormalities in regulators of plasminogen activation or plasmin degradation have been reported as causes of familial bleeding disorders (eg, alpha-2 antiplasmin deficiency, plasminogen activator inhibitor-1 deficiency). Mild hemophiliac patients with factor activity levels above approximately 15 to 20 percent are often sufficient to prevent spontaneous bleeding and to produce a normal PT and aPTT. However, patients with mild deficiency of

Table 4: Genetics of common bleeding disorder

Disorder	Genetics
Hemophilia A (Factor VIII deficiency	Sex-linked recessive Xq28
Hemophilia B Christmas Disease (Factor IX deficiency)	Sex-linked recessive Xq27.1-27.2
Von Willebrand Disease (Most common disorder of platelet function, many variants (Type I-III, Pseudo, etc.) manifestations mild to severe	Autosomal dominant, recessive, X -linked
Thrombasthenia (Impaired platelet function) Glanzmann, Bernard-Soulier disease	Autosomal recessive
Owren's disease / Parahemophilia (Factor-V deficiency)	Autosomal recessive 1q21-25
Factor-VII (Proconvertin	Autosomal recessive 13q34
(Factor I deficiency) Afibrinogenemia/ Hypofibrinogenemia/ Dysfibrinogenemia	Autosomal recessive 4q23-34

a coagulation factor may have increased bleeding with hemostatic challenges (eg, excessive surgical bleeding, menorrhagia). This may be seen in an individual who is heterozygous for a coagulation factor defect, such as a hemophilia carrier.

Lastly, hemostatic disorders resulting due to structural abnormalities (eg, hereditary hemorrhagic telangiectasia), hereditary disorders of connective tissue (eg, Ehlers-Danlos disease, osteogenesis imperfecta), acquired connective tissue disorders (eg, scurvy, steroid-induced purpura), small vessel vasculitis, and purpura associated with the presence of paraproteins will also have a normal screening coagulogram.

Some patients are encountered with a significant bleeding history for which there is no explanation. Abuse, occasionally self-inflicted, should be considered.

GENETICS OF BLEEDING DISORDERS

Ever since the cloning and characterization of the first coagulation factor gene in 1982, considerable progress has been made in the use of molecular genetic strategies to assist in diagnosis of bleeding disorders. Working up on the mutational data base also helps in knowing the genetic basis of the coagulation disorders within a region and helps in determining management and prevention strategies[10,11]. A study from western part of India reported on 630 patients, diagnosed to have hereditary bleeding diathesis[12]. Amongst these, 598 (95%) patients had a coagulation disorder and 32 (5%) patients had a platelet function abnormality. In a group of coagulation disorders, hemophilia A (70.5%) was the most common disorder followed by hemophilia B (14%) and von Willebrand disease (10.8%). However Glanzman's thrombasthenia (84.3%) was found to be the most common platelet function disorder followed by Bernard-Soulier syndrome (12.5%). Table 4 summarises the common bleeding disorders and their genetic basis.

A number of mutation screening methods have been used for detecting mutations, which include denaturing gradient gel electrophoresis (DGGE), single strand conformational polymorphism (SSCP), conformational sensitive gel electrophoresis (CSGE) and chemical cleavage mismatch (CMC). These result in a mutational detection rate up to 90%. All 1st degree female relatives of severe and moderate hemophilia must get factor assays done because some of them may be vulnerable to post-procedural or post-traumatic bleeding.

CONCLUSION

The approach to a patient with a bleeding disorder needs a comprehensive detailed history and thorough physical examination. There must be a logical systematic approach and a discriminate use of laboratory investigations to reach the diagnosis and assess severity. Particular emphasis should be placed on family and drug history. A simple approach to detect the cause is to look at the hemostatic system as three compartments- blood vessels, platelets and coagulation proteins. Genetic workup should be done whenever feasible to personalize the management of the patient.

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