Coagulation, bleeding and thrombosis

Summary prepared by: Amr Qudeimat

We'll start with a quick review of relevant physiology. Hemostasis is divided into primary and secondary hemostasis. Primary hemostasis relies on normal subedothelium, platelets and vWF. Platelet and vWF disorders can be either quantitative or qualitative. Secondary hemostasis depends on coagulation factors and vWF. There are many coagulation factors. Factor I is fibrinogen. factor II is prothrombin. The numbering goes all the way to XIII and finally, we have vWF. All coagulation factors are made in the liver except for vWF (endothelial cells and megakaryocytes) some factors are synthesized in the liver in addition to another site (factor V megakaryocytes, factor VIII endothelia cells and factor XIII macrophages. All coagulation factors have lower levels in infants with the exception of I, VIII and vWF. It's important to recognize that factor VII has the shortest half-life of 3-6 hours (earliest to drop in liver failure) and factor XIII has the longest half-life of 10 days which correlates with its function in wound remodeling. Also, recognize vitamin K dependent coagulation factors (II, VII, IX, X). Factors VIII, vWF and fibrinogen are also acute phase reactants.

The final steps in the coagulation cascade involves the conversion of fibrinogen to fibrin with the help of thrombin and then the cross linking of fibrin through the action of activated factor XIII. Since fibrinogen is an acute phase reactants. This means that inflammatory states it's serum level may over estimate actual reserve. Elevated fibrinogen levels also result in higher ESR reads even in the absence of inflammation. Testing for fibrinogen deficiency can be done through sending PT, PTT, TT and measuring functional fibrinogen. If the patient is concurrently on heparin, send a reptilase time.

Factor XIII also has special importance. It's the factor with the longest half life which is justified by it's central function in wound healing and remodeling. Factor XIII deficiency can result in severe delayed bleeding but with normal PT and PTT. Common associations are umbilical stump bleeding and delayed separation in addition to intracranial bleeding.

Fibrinolysis and natural coagulation inhibitors also have a central role in this process. Both tPA and uPA activate plasminogen to plasmin which dissolves a fibrin clot giving fibrin split products and D-dimer. Plasmin is inhibited by both alpha-2 antiplasmin and thrombin activated fibrinolysis inhibitor. Testing the activity of the fibrinolytic system is through measuring fibrin split products and D-dimer in the blood. tPA and uPA are available in drug form for the treatment of arterial and venous thrombosis in addition to central venous line occlusions. Drugs to inhibit the fibrinolytic system by blocking plasmin's binding site for fibrin are also available (aminocaproic acid and tranexamic acid).

Vitamin K dependent coagulation factors include factors II, VII, IX and X in addition to protein C and protein S. they need vitamin K to be carboxylated to the active form. This is blocked by the action of vitamin K antagonists such as warfarin. Warfarin's therapeutic effect can be monitored with INR. Since this both depends on factor VII which has a very short half life and factor X that has a very long half-life, a rise in INR represents the depletion of factor VII while an anti-coagulated state is not achieved yet. It takes at least 5 days for the full effect to takes place during which time the patient should be on another form of anti-coagulation. Overlap should be for at least 2 days after INR is therapeutic. Warfarin has a narrow therapeutic index and many drug and food interactions and should never be admitted to pregnant women due to the risk of warfarin embryopathy. It can be revered by vitamin K, FFP and prothrombin complex concentrates. Therapeutic INR is generally between 2-3 with higher values such as 2.5-3.5 used in cases like prosthetic valves and antiphospholipid syndrome. Since proteins C and S are both vitamin K dependent, skin necrosis may happen early after the initiation of warfarin therapy as protein C is depleted very early on. Desmopressin (DDAVP) has effects on several of the elements involved in the coagulation cascade. It increases serum levels of vWF, FVIII (so it can be used to treat mild hemophilia cases) and tPA release.

PT, PTT and TT are prone to many testing problems resulting in inaccurate results. The test relies on a specific ratio between plasma and anticoagulant in the tube so underfilling the tube will result in overestimation and vice versa. Anemia means there will be relatively more serum in the tube resulting in lower values too. Hard stick also results in factor consumption in vitro and underestimation of these parameters. Not processing samples in a timely manner in the lab can

also affect results. Remember that PT and PTT screen mainly for factor deficiencies and not bleeding tendency (FXIII deficiency has severe bleeding but normal PTT and PT, FXII deficiency has no bleeding but abnormal PTT).

A condition that is worth reviewing here is DIC, which occurs as a secondary result to another process such as trauma, stress, infection, malignancy or severe hemolysis). While bleeding or thrombosis may not be present in mild DIC, both can occur simultaneously in more severe cases due to consumptive coagulopathy. Hemolytic anemia and end organ damage can also occur due to DIC. There is not a specific test for diagnosing DIC but usual lab findings include elevated PT and PTT, thrombocytopenia, low fibrinogen, elevated FDPs and D-dimers in addition to low proteins C and S. DIC in neonates can present with purpura fulminans characterized by skin necrosis due to macrovascular thrombosis secondary to low proteins C and S in this age group. Treatment relies on addressing the underlying cause and supportive measures like FFP and cryoprecipitate infusions. Platelet transfusions addressing the thrombocytopenia cause thrombosis and are not recommended.

Vitamin K deficiency is a significant issue in neonates who do not receive vitamin K prophylaxis shortly after birth. Classically it occurs at 2-7 days post birth can present early in the first 24 hours if the mother was on a vitamin K antagonist during pregnancy and late after 1 month if the patient has a condition that interferes with vitamin K metabolism such as cystic fibrosis. All forms can present with GI and intracranial bleeding but the late form wont present with umbilical site bleeding. All cases are treated with vitamin K supplement and response time is typically less than 24 hours. Prothrombin complex concentrates can be used in acute severe bleeding like ACH but carry the risk of thrombosis. Vitamin K deficiency can also occur due to pesticide exposure that contains warfarin like toxin. These toxins, though, have a much longer duration of action and require prolonged high dose vitamin K therapy.

Liver disease related bleeding occurs mainly due to the fact that most clotting factors are made by the liver. This is exacerbated by thrombocytopenia due to hypersplenism and toxins effect on the bone marrow and thrombopoietin deficiency. PT is typically elevated first due to the short half-life of factor VII and PTT follows as liver dysfunction worsens. Fibrinogen may be elevated early in liver disease as it's an acute phase reactant and it drops later as liver status worsens since it's made in the liver. D dimers are also elevated (removed by the liver). Fresh frozen plasma (for replacement of factors) and cryoprecipitate (for replacement of fibrinogen) in addition to platelet transfusions are indicated. Acute bleeding episodes warrant factor VII infusions. Prothrombin complex concentrates may also be considered but may carry a significant thrombosis risk.

Mixing studies can help delineate the etiology of an elevated PTT. If mixing the patient's sample with normal plasma corrects the elevation in PTT the underlying cause is a factor deficiency and if not, it's related to an inhibitor. The classic example of inhibitor is in hemophilia patients receiving factor replacement therapy, but a more common cause encountered is lupus anticoagulant. Most lupus anticoagulants are transient, asymptomatic and result from an acute viral infection (one of the most common causes of a prolonged PTT). Lupus anticoagulants can be part of the antiphospholipid syndrome and cause thrombosis. Rarely, patients with the lupus anticoagulant also have acquired hypoprothrombinemia (due to increased clearance of prothrombin) which can result in bleeding (known as "Lupus anticoagulant hypoprothrombinemia syndrome"). Once we have a prolonged PTT (or rarely PT) that doesn't correct with mixing study, this triggers running assays for specific factor inhibitors and the dilute Russell's viper venom time (dRVVT) test looking for lupus anticoagulant. Most lupus anticoagulants are transient and asymptomatic and can be managed by observation; Repeat assay in 6-12 weeks to document resolution with no specific therapy indicated. If bleeding due lupus anticoagulant hypoprothrombinemia syndrome occurs, replace prothrombin with prothrombin complex concentrates. Thrombosis in antiphospholipid antibody syndrome is managed with anticoagulation. Other acquired coagulation disorders include: 1) Hemophagocytic lymphohistioctyosis syndrome which can have severe coagulopathy with hypofibrinogenemia. 2) Treatment with asparaginase. Asparagine is important for many coagulation proteins. Patients can have thrombosis from decrease in antithrombin, proteins C and S. 3) Nephrotic syndrome: Leads to loss of antithrombin in the urine. Increases the risk for thrombosis. 4) Protein losing enteropathy: Loss of proteins C/S and antithrombin with increased risk for thrombosis.

Hemophilia describes either factor VIII (A) or IX (B, Christmas disease) inherited deficiency. Both are inherited in an X-Linked recessive manner. Please note that rarely, female carriers/ heterozygotes can still present with mild to moderate Hemophilia due to non-random X chromosome deactivation. Many mutations can result in this disease. Milder disease is usually linked to point mutations while severe disease tends to result from larger deletions. The severity of the phenotype depends on the residual factor activity where less than 1% presents with severe disease, 1-5% moderate and 5-40 with mild disease. Presentation is usually a joint bleed (hemophilic arthropathy), post circumcision or post vaccination bleeding, or a post-surgical bleeding usually with a positive family history. Intracranial bleeding can also occur. Severe disease usually shows itself in the first few months of life with mild disease can take more than a decade to present. The more severe the disease, they more likely it will develop an inhibitor in its course. Hemophilia B is also less likely to develop an inhibitor than A. A common type of internal hemorrhage, retroperitoneal hemorrhage usually results from bleeding in the iliopsoas muscle. Patients present with pain in flank, groin or rarely the thigh. Difficulty walking is typical where a patient walks hunched over to relax the iliopsoas. PTT is almost always prolonged and factor assays show the deficient factor and residual activity level. Gene analysis studies can also be utilized to show specific factor mutations. Management is based on the deficient factor replacement in addition to using DDAVP (helps some patients with mild disease) and antifibrinolytics for mucosal bleeding. Factor replacement is usually administered prophylactically with primary prophylaxis meaning the initiation of factor replacement prior to any joint bleeding, secondary prophylaxis meaning the initiation of factor replacement after the onset of joint bleeding and before arthropathy in order to prevent further bleeding and joint disease and tertiary prophylaxis being the initiation of factor therapy after onset of arthropathy to prevent progression. Prophylaxis is usually indicated in all severe patients and some moderate patients. Synovectomy may be helpful in a joint that develops chronic arthropathy as it helps improve joint movement, reduce pain and improve function. It usually takes several bleeds into a joint to develop arthropathy.

Desmopressin increases FVIII levels in most patients with mild hemophilia A by increasing circulating vWF. It's not helpful in FIX deficiency. It's generally used for mucocutaneous bleeding and minor procedures (dental). Some mild mutations are not responsive so the patient must undergo a desmopressin challenge before prescribing it. Patients less than 3 years of age can develop hyponatremia with this modality and some patients may develop hypotension and flushing for which the first dose should be administered in the clinic under observation.

Before surgery: ensure the patient does not have an inhibitor and replace factor to 100% immediately prior to procedure. Repeat bolus doses to maintain a trough activity level of >50% until bleed risk has passed. Antifibrinolytic drugs can be added especially for mucus membrane surgery. Before dental extractions: Correct to 80-100% and add a antifibrinolytic agent as aminocaproic acid or Tranexamic acid and use pressure gauze post procedure. For patients with hematuria: correct factor level to 80-100%, use hydration (1.5-2x maintenance) to prevent clotting in the urinary tract. Antifibrinolytic agents are contraindicated due to risk of clotting.

Failure of factor replacement therapy is usually due to inadequate dosing or the development of an inhibitor. An inhibitor is a neutralizing antibody that renders factor replacement less effective or ineffective. Inhibitors significantly complicate the management of hemophilia as bleeds become more difficult to treat and prophylaxis becomes not as effective. Inhibitor patients have worse morbidity. If this occurs, send testing for inhibitors. Patients with inhibitors need a bypassing agent to control bleeding (FVII). Prophylactic therapy should be put on hold. Risk Factors for the development of an Inhibitor are family history of inhibitors, more severe hemophilia, certain molecular defects (Large deletions>Nonsense mutations>Inversions>Missense mutations) and race (African>Caucasian). Management of inhibitors relies on immune tolerance therapy, the use of a bypassing agent (factor VIIa, activated prothrombin complex concentrates) and using higher doses of factor VIII or IX in patients with low inhibitor titers.

Von Willebrand disease is the most common bleeding disorder. It usually presents with mucocutaneous bleeding except type 3 (Type 3 have mucocutaneous bleeding and hemophilia-like bleeding). Typical symptoms are: Epistaxis, easy bruising, oral bleeding, post-surgical (oropharyngeal) bleeding and menorrhagia and post-partum bleeding in females of childbearing age. Von Willebrand factor's function is Platelet binding and it's a Carrier molecule FVIII in the circulation. vWF is increased by physiologic stress, DDAVP, estrogen, pregnancy, and is an acute phase reactant. There are several types of vWD:

A) Type 1 (autosomal dominant): Heterozygous defect with reduced production of normal vWF.
B) Type 2A (autosomal dominant): Multimerization defect with absent large/intermediate size multimers.
C) Type 2B (autosomal dominant): Gain of function mutation in VWF (too adherent to platelets so large multimers are attached to platelets and not circulating).
D) Type 2M (autosomal dominant): Loss of function mutation (opposite of 2B), vWF doesn't bind well to platelets.
E) Type 2N (autosomal dominant): Loss of vWF binding function to FVIII.
F) Type 3 (autosomal recessive): Absence of vWF production.

Be familiar with types I and III. Differentiating the subtypes of type II are more for the specialty level and are mentioned here for continuity. Also, notice that only type III is autosomal recessive and the rest are dominant. Diagnostic workup includes vWF antigen level, vWF multimer analysis, FVIII activity level and platelet aggregation studies. There are several treatment options. We can try to increase circulating vWF with Desmopressin (DDAVP) administration. It helps release stored vWF from Weibel-Palade bodies in endothelium. Another option is to replace with plasma-derived vWF concentrate. We can also inhibit fibrinolysis with antifibrinolytics. Hormonal therapy may be effective for menorrhagia management as estrogen increases vWF and FVIII and reduces blood flow to endometrium. Topical therapy with antifibrinolytics can be used for oral bleeding and topical thrombin for oral or nose bleeding.

Dysfibrinogenemia is a rare inherited disorder that develops due to a dysfunctional fibrinogen. It Can cause bleeding or thrombosis (Bleeding due to dysfibrinogens with loss of proper clot forming structure and thrombosis due to dysfibrinogens which resist fibrinolysis). Lab findings include: prolonged thrombin time and an abnormal ratio of fibrinogen antigen to fibrinogen function. Treatment is by replacing fibrinogen with plasma derived fibrinogen concentrate or cryoprecipitate. If thrombosis occurs, anticoagulation is initiated.

Prolonged thrombin time is usually due to absent or very low fibrinogen, dysfibrinogenemia or heparin contamination. First, ensure that there is no heparin contamination and if there is none, measure functional fibrinogen. If normal, measure fibrinogen antigen and function together to assess for dysfibrinogenemia. Normal antigen and reduced function is suggestive of dysfibrinogenemia. For patients on heparin, measure the Reptilase time; it assesses fibrinogen conversion to fibrin (like thrombin time) and it's resistant to the effects of heparin.

The following paragraphs, will include a short description of anticoagulants and anticoagulation therapy. Detailed description of thrombotic disorders won't be included here but a few important aspects of them will be mentioned.

Natural Anticoagulant proteins (protein C, protein S and anti-thrombin) are reduced at birth. This means we must interpret results in first year of in the context of age appropriate values. The liver produces most of these factors so, liver disease leads to deficiency and decreased clearance of activated factors increasing risk of coagulopathy and thrombosis.

Thrombophilia is a predisposition to develop thrombi. Examples of venous thrombotic events (VTE) are: deep vein thrombosis (DVT), pulmonary embolism (PE), cerebral Sino venous thrombosis, renal vein thrombosis, portal vein thrombosis, and mesenteric. Arterial events can also occur such as arterial ischemic stroke and catheter-related events.

Examples of predisposing factors are:

A) Inherited: antithrombin deficiency, protein C deficiency, protein S deficiency, factor V Leiden mutation, prothrombin G20210A mutation, dysfibrinogenemia, hyperhomocysteinemia, elevated FVIII, elevated lipoprotein A.
B) Acquired: antiphospholipid antibody syndrome (APS), anticardiolipin, anti-β2 glycoprotein, lupus anticoagulant and some medical conditions such as malignancies, bed ridden state etc.

Antithrombin is made in liver. It neutralizes thrombin (IIa), Xa, and IXa. It reaches adult levels by 12 months of age. Heparin potentiates its activity. It has two binding sites; Heparin binding site and a substrate binding site (reactive) center. Deficiency can be congenital or acquired such as: DIC, sepsis, burns, trauma, liver disease, nephrotic syndrome (protein loss) or heparin (accelerated clearance). AT deficiency results in thrombotic events, venous events more common

including DVT, PE and Mesenteric. 40% of events are spontaneous and have a high rate of PE and a very high rate of recurrence. Homozygous state results in fetal demise.

Protein C is a vitamin K dependent protein produced in liver that needs to be activated by thrombin/thrombomodulin complex. Deficiency follows and autosomal dominant pattern. Protein C levels are low in infancy and don't normalize until adolescence. Acquired Causes of deficiency are Liver disease, DIC, Sepsis and Uremia. It carries a 70% spontaneous thrombosis risk, mainly venous with high risk of recurrence. It can result in Purpura fulminans, ophthalmologic injury, renal injury, and neurologic complications in neonates and requires anticoagulation for life.

Protein S is another vitamin K dependent protein and is also produced in liver, endothelial cells, platelets and brain cells. It reaches adult levels by 12 months of age. In addition to rare congenital deficiency, acquired causes include estrogen, OCPs, Pregnancy, DIC, Liver disease, Acute thrombosis and Inflammatory states. Majority of cases are venous with high recurrence rates.

Factor V Leiden mutation increases the risk for venous thrombosis. It causes activated protein C resistance. If the mutation is heterozygous the risk of recurrence is low. Antiphospholipid syndrome has persistent presence of antiphospholipid antibodies or lupus anticoagulant for ≥ 12 weeks in context with an acute thrombotic event. Venous or arterial thrombosis can occur, recurrent pregnancy loss < 10 weeks of gestation and premature delivery or fetal demise >10 weeks of gestation.

Heparin binds to antithrombin (cofactor) increasing antithrombin's inhibition of thrombin (factor IIa) and factors IXa, Xa, XIa, XIIa, and kallikrein. It binds non-specifically to other plasma proteins and platelet derived proteins. It has a short half-life, can be reversed with protamine sulfate and is excreted by kidney. There is a risk of developing heparin induced thrombocytopenia (HIT). aPTT is very sensitive to heparin contamination. Monitoring is commonly done by PTT (Target range 65-80 seconds or 1.5-2.5X baseline). Measure every 4 hours and once therapeutic, daily.

Direct thrombin inhibitors are not commonly used in pediatric patients unless in cases like heparin induced thrombocytopenia where alternatives to both unfractionated and low molecular weight heparin need to be used. Most of them have a very short half- life (around 1 hour or less) and most don't have antidotes.

Low molecular weight heparin works though AT mediated, Xa and partial IIa inhibition. It has <50% the molecular weight unfractionated heparin thus Reducing anti-IIa activity due to its small MW. It carries Less risk of HIT and is partially reversible with protamine sulfate. It's renally cleared so contraindicated in renal failure and needs dose reduction in renal insufficiency. Levels are obtained 4-6 hours after administration with a goal 0.5 to 1.0 U/ml for treatment. Fondaparinux works by AT mediated selected inhibition of Xa, is renally and is not reversible.