CLINICAL VIGNETTE

Approach to the Patient with an Isolated Prolonged Activated Partial Thromboplastin Time: Etiology, Diagnosis, and Management in Three Cases

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Case 1: Isolated aPTT prolongation presenting without associated hemorrhage or thrombus

A 65-year-old male with pancreatic cancer metastatic to the liver was evaluated for atypical chest pain during a chemotherapy infusion of gemcitabine through a totally implantable vascular device (port). Physical examination was unremarkable, and acute coronary syndrome and pulmonary embolism were excluded. Initially, laboratory data was significant for PT/aPTT//INR: 13 s/124 s/1.0, drawn from a port. The specimen was redrawn through a peripheral vein with PT/aPTT/INR values of 12 s/29 s/1.1. One week prior to presentation PT/aPTT/INR had been 13 s/28 s/1.0. Hemoglobin was stable and platelet count normal.

The aPTT prolongation resulted from specimen contamination with heparin used to flush the port.

Case 2: Isolated aPTT prolongation presenting with associated thrombus

A 75-year-old female with breast cancer, status post left modified radical mastectomy and chemotherapy, followed by 5 years of tamoxifen completed 5 years prior, presented with left arm pain. Physical examination was significant for tenderness over the left biceps muscle. A left upper extremity Doppler ultrasound demonstrated a left brachial vein deep venous thrombus. Laboratory data revealed a normal CBC. PT/aPTT/INR were 13.6 s/62.7 s/0.98 on presentation and 12.8 s/58.1 s/0.96 one year prior. Mixing test showed no significant correction in the aPTT (61 s).

The aPTT was prolonged because of the presence of a lupus anticoagulant. The patient was anticoagulated with an INR goal of 2-3.

Case 3: Isolated aPTT prolongation presenting with associated hemorrhage

A 52-year-old female with temporal lobe epilepsy, status post temporal lobe resection 4 years prior, and sarcoidosis associated with mediastinal lymphadenopathy was seen after she noted a large hematoma involving her right hand that developed spontaneously over 24 hours. She also reported easy brusability for 2 months. She was maintained on zonisamide and levetiracetam for seizure prophylaxis after developing a drug rash from phenytoin. Physical exam revealed an expanding soft tissue hematoma over the dorsal aspects of the 3rd, 4th, and 5th digits and palm of her right hand, and scattered 2-3 cm ecchymoses involving her bilateral upper and lower extremities.

Laboratory data: Hgb 15.7 g/dL, platelets 242 x 103/μL, stable from 5 days prior; PT/aPTT/INR: 12.8 s/64.3 s/0.91 compared to 12.6 s/28.9 s/0.89 two years prior; liver function tests: AST 163 U/L (10-37 U/L), ALT 152 U/L (5-37 U/L), alkaline phosphatase 149 U/L (33-97 U/L), total bilirubin 0.9 mg/dL (0.2-1.2 mg/dL); five days prior: AST 49 U/L, ALT 36 U/L, alkaline phosphatase 81 U/L, total bilirubin 1.2 mg/dL.

Mixing test: 12.8 s/40.3 s/0.91 (aPTT partially corrected after a 60 minute incubation). Factor level assays: II, IX, XI, XII normal. Factor VIII: 2% (46-151 % of normal). Factor VIII inhibitor screen (Bethesda assay): >20 Bethesda units (normal: <5 BU).

The aPTT prolongation was due to an acquired Factor VIII inhibitor attributable to sarcoid with hepatic involvement. The patient was treated with 2 units of fresh frozen plasma, but developed an acute urticarial reaction during the infusion. Prednisone and cyclophosphamide were started with a post-treatment aPTT of 46.6.

Discussion

The activated partial thromboplastin time (aPTT or PTT) is an *in vitro* assessment of the intrinsic and common pathways of the classical waterfall-cascade model of coagulation. Although it does not reflect *in vivo* physiology depicted in the current revised model, the aPTT remains a practical and effective laboratory screening test[1-4].

The Classical Waterfall-Cascade (In Vitro) Model of Coagulation

In the classical waterfall-cascade model of blood coagulation, the extrinsic pathway, initiated by tissue factor[5], and the intrinsic pathway, initiated by contact activation[6], are parallel enzymatic systems that converge on a common pathway mediated by factor Xa. The common pathway culminates in the conversion of prothrombin to thrombin (factor II to factor IIa), which converts fibrinogen (factor I) to insoluble fibrin on the platelet surface, stabilizing the existing clot[2,7].

The extrinsic pathway is triggered by exposure of tissue factor to plasma during injury. Tissue factor (TF), also known as factor III or tissue thromboplastin[7], is an integral membrane glycoprotein constitutively expressed on subendothelial fibroblasts and smooth muscle cells of the vessel wall[1-3]. Tissue factor has also been identified intravascularly in circulating monocyte and platelet derived microparticles[1] with latent or encrypted procoagulant activity[3], that putatively contribute to thrombotic disease states[3,7,8].

After exposure to plasma in the presence of calcium[2], tissue factor binds to factor VIIa (1% of circulating factor VII) or to factor VII proper, which it converts to factor VIIa, producing the most potent activator of coagulation known[4]. The resulting factor VIIa/TF complex subsequently activates factor X to Xa either directly (via the extrinsic pathway) or indirectly through the conversion of factor IX to IXa, using factor VIIa[1-3] in contact with calcium and platelet membrane phospholipids (intrinsic pathway)[2]. Factor Xa production marks entry into the common pathway, which requires factor Va to convert prothombin to thrombin[1-3].

Historically, the intrinsic pathway is termed the contact activation pathway because it is activated *in vitro* by negatively charged surfaces[6,9], as observed when factor IX is exposed to glass or kaolin[2], or when factors XI and XII (Hageman factor) and plasma proteins prekalikrein and high molecular weight kininogen, which represent the "intrinsic" components of the blood[10], are exposed to subendothelial tissue during injury[1,2,6]. However, deficiencies in factor XII, prekalikrein, and high molecular weight kininogen do not result in bleeding disorders, making the physiological role of these proteins unclear and resulting in their omission from revised coagulation schemes[2].

The Revised (In Vivo) Model of Coagulation

In the revised model of coagulation (**Figure 1**), the intrinsic pathway is not required for *in vivo* initiation of coagulation[3], and the two pathways occur in series[7]: the extrinsic pathway initiates clotting via tissue

factor and activates the intrinsic pathway through the production of factor IXa, and by the generation of a small quantity of thrombin (insufficient to complete fibrin formation)[11] that primes the system and positively feeds back on factors VIII, V, and XI[3,6,9]. The intrinsic pathway, in turn, amplifies the system in a burst of thrombin production that propagates both fibrin formation and factor XIIIa-dependent fibrin crosslinking[1-4]. Soon after initiation, the protease inhibitor tissue factor pathway inhibitor (TFPI) inhibits the extrinsic pathway, so that the majority of thrombin is generated through the intrinsic pathway amplification loop[2,12].

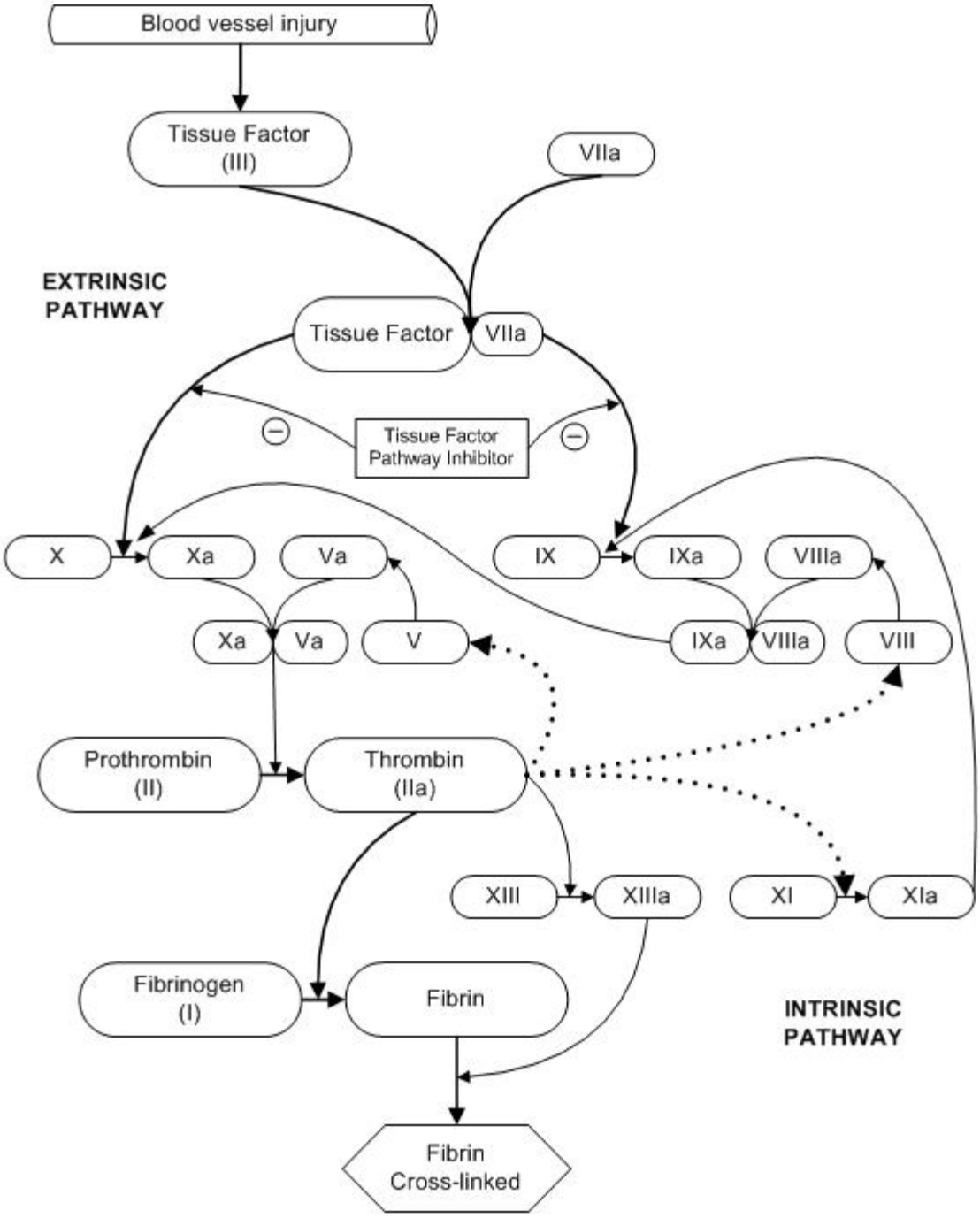

Figure 1. The revised model of coagulation.[1-3]
After activation by tissue factor, the extrinsic pathway is quickly inhibited by tissue pathway factor inhibitor, so that thrombin production is amplified through factors V, VIII, and XI (dotted arrows).

The Activated Partial Thromboplastin Time

The aPTT assesses intrinsic pathway factors VIII, IX, and XI, common pathway factors I, II, V, and X, as well as prekalikrein, high molecular weight kininogen, and factor XII, whereas, the prothrombin time (PT) assesses the extrinsic and common pathway factors VII, I, II, V, and X[1].

The aPTT quantifies the time in seconds required for clot formation after phospholipid (an intrinsic pathway activator) and calcium are added to plasma. Since thromboplastin is comprised of both phospholipids and tissue factor, the term "partial" thromboplastin time reflects the absence of tissue factor in the aPTT reagent[13]. The reference range for the aPTT depends upon the reagent-instrument combination used; thus, each laboratory establishes its own range using healthy subjects[14]. The approximate lower and upper limits of normal for the aPTT are 20-25 seconds and 32-39 seconds, respectively[15].

An isolated aPTT prolongation (i.e., with a normal PT and platelet count) indicates derangement in the intrinsic pathway and warrants further evaluation.

Etiology and Management of Isolated aPTT Prolongation

Isolated prolongation of the aPTT may be inherited or acquired. Inherited causes include: 1) *intrinsic pathway deficiencies with associated bleeding diatheses* (factors VIII, IX, and XI [hemophilias A, B, and C, respectively])[13], and *without bleeding diatheses* (factor XII, prekalikrein, and high molecular weight kininogen)[2,15]; and 2) *Von Willebrand disease*, which reflects a functional deficiency in factor VIII, because von Willebrand factor (vWF) significantly prolongs factor VIII survival in plasma as the factor VIII carrier protein[16]. Factor deficiencies of 15 –50% are necessary to prolong the aPTT[1,15].

Acquired etiologies include: 1) *laboratory error or artifact*, 2) *exogenous anticoagulants,* 3) *lupus anticoagulants (nonspecific inhibitors)*, and 4) *specific inhibitors* of factors VIII, IX, XI, and XII, and vWF[1,13].

Laboratory error or artifact: Specimens should be assayed within 4 hours of collection, as factor VIII lability may prolong the aPTT thereafter. During collection for aPTT analysis, patient samples are added to citrated anticoagulant in a ratio of whole blood to anticoagulant of 9:1, and plasma to anticoagulant of 5:1. Both erythrocytosis and inadequate filling of the collection tube will decrease the plasma: anticoagulant ratio, leading to over anticoagulation and artifactual prolongation of the aPTT[13]. When unfractionated heparin is present in the specimen, either therapeutically or as a contaminant from a sample obtained through a heparin-flushed catheter, heparin-induced antithrombin-dependent inactivation of thrombin will prolong the aPTT[17] (as in **Case 1**). One study showed that heparin contamination accounted for 39% of unexpected aPTT prolongations[18]. Low-molecular-weight heparins rarely prolong the aPTT greater than 40 seconds[13].

Lupus anticoagulants are antiphospholipid antibodies that inhibit phospholipid-dependent *in vitro* coagulation reactions, prolonging the aPTT, but paradoxically increasing the risk of venous and arterial thromboembolism[15,19,20] (odds ratio of 11)[21] through an undefined mechanism[19]. In **Case 2** the patient presented with an upper extremity DVT, which represents 1-4% of all DVT cases, and in a recent study, the most commonly associated acquired thrombophilia in upper extremity DVT was lupus anticoagulant (31%)[22]. Persistence of lupus anticoagulant in conjunction with thrombosis constitutes the anitphospholipid syndrome, which requires chronic anticoagulation[20]; an INR goal of 3.1 to 4.0 is not superior [23,24] to 2.0 to 3.0.

Factor inhibitors occur rarely. Factor VIII inhibitor (acquired hemophilia A) is the most common type[15] with an incidence of 1 to 4 per million/year[25] and a median age of 60-77 years[26,27]. Acquired hemophilia A (AHA) results from development of an autoantibody to factor VIII[25,26]. Up to 50% of cases are idiopathic; other etiologies include autoimmune disease (such as sarcoidosis in **Case 3**), malignancy, lymphoproliferative disorders, postpartum states, drug reactions (phenytoin allergy is in the differential diagnosis of **Case 3**) [26,28], or reactions to factor VIII infusion (seen in 10% to 20% of patients with severe congenital hemophilia A)[15]. AHA is associated with widespread, often spontaneous, subcutaneous, soft tissue, and mucosal bleeding; but hemarthroses, seen in congenital hemophilia A, are uncommon[26-28]. Severe bleeds occur in as many as 90% of patients, with fatal hemorrhage in 9-22% of cases[25]. However, severity of bleeding does not correlate with inhibitor strength or factor VIII levels[27].

Goals of treating factor VIII inhibitors include: 1) *control of the bleeding*[25,26] and 2) *eradication of the inhibitor*[25-27]. Factor VIII inhibitor bypassing agents (recombinant factor VIIa and activated prothrombin complex concentrate [APCC]) are first-line treatments to control bleeding with high titers of inhibitor (>5 Bethesda units [BU]) [25,26], and desmopressin (DDAVP) and factor VIII human or porcine concentrates are used for low titers (< 5 BU)[25,26]; porcine concentrates are advantageous because they demonstrate less cross reactivity with the inhibitor[26]. Eradication of the inhibitor is achieved with either corticosteroids alone or corticosteroids and cyclophosphamide, although neither strategy has demonstrated a superior outcome. Rituximab and cyclosporine are 3rd line agents; immunoadsorption or plasmapheresis may be used when available. Relapse in AHA occurs in 20% of patients, mandating close follow up[27].

Evaluation of an Isolated Prolonged aPTT

1. ***Evaluation of the specimen***. To determine whether the specimen was correctly collected and stored, or if it has been artifactually prolonged even by trace amounts of heparin in a central line[15], repeat cannulation via a peripheral line can be performed. Alternatively, heparinase addition to the specimen will neutralize exogenous heparin, and the aPTT will approximate that of a peripheral line specimen[29]. Heparin may also be detected by checking the thrombin time (clotting time after addition of thrombin) versus the reptilase time (clotting time after addition of thrombin-like pit viper snake venom). Heparin will prolong the thrombin time but does not change the reptilase time because, in contrast to thrombin, pit viper venom is unaffected by heparin[10].

2. ***Use of the PTT mixing study***. The aPTT mixing study distinguishes between a factor deficiency and the presence of an inhibitor.

Normal pooled plasma and patient plasma are mixed in a 1:1 ratio, and the aPTT is measured immediately, and after incubation at 37^{o}C for 30, 60, and/or 120 minutes[1].

The aPTT will correct with mixing and remains corrected after incubation with isolated factor deficiencies or von Willebrand disease. To characterize the deficiency[1,13] individual factor assays for factors VIII, IX, XI, and XII should be performed using a modification of the mixing study. Von Willebrand disease is confirmed by reductions in factor VIII activity and by two additional studies: the ristocetin cofactor assay and von Willebrand factor antigen assay, which assess vWF function and quantity, respectively[15].

If the a*PTT does not correct with both mixing and incubation*, an inhibitor commonly, lupus anticoagulant is present[1,13,15]. Confirmation of lupus anticoagulant requires: 1) prolongation of at least one phospholipid-dependent coagulation assay (dilute Russell's viper venom time (DRVVT), kaolin clotting time (KCT), dilute thromboplastin time, dilute aPTT, dilute prothrombin time, and platelet neutralization

procedure (PNP)[15]; and 2) reversal of inhibition of *in vitro* coagulation after the addition of phospholipid[13].

If the aPTT corrects initially, but becomes prolonged after a 1 or 2 hour incubation period, a factor VIII inhibitor is suggested[1,13,25]. A factor VIII assay is then performed, and if low, lupus anticoagulant, which may artifactually lower factor VIII levels, should be excluded[27]. A decrease in the factor VIII assay, usually to less than 10% of normal, necessitates a factor VIII inhibitor screen (Bethesda assay)[15]. The Bethesda assay confirms the presence of a factor VIII inhibitor and measures its strength[27], providing data to guide treatment. Serial dilutions of patient plasma are performed to dilute the inhibitor. The dilution inhibiting 50% of factor VIII in normal controls represents the titer of the inhibitor in Bethesda units; the greater the Bethesda units, the stronger the inhibitor. Other specific factor inhibitors can be identified and tittered by modifying the Bethesda assay accordingly[15].

Summary

An isolated prolonged aPTT warrants further investigation. Once artifact or heparin use is excluded (**Case 1**), the test of choice in determining the etiology is the mixing test, which distinguishes between factor deficiency and the presence of an inhibitor. In asymptomatic patients or those with thrombosis (**Case 2),** lupus anticoagulant should be excluded. In patients with bleeding manifestations (**Case 3**), assays for factor deficiencies or inhibitors are indicated.

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