Evaluation of a Prolonged Prothrombin Time

Joshua L. Hood and Charles S. Eby*

CASE DESCRIPTION

A 47-year-old African American woman was evaluated for a prolonged prothrombin time (PT) result obtained before she underwent right total hip arthroplasty. The patient had no history of gastrointestinal or intracranial bleeding, epistaxis, or hemarthrosis. However, she reported a tendency toward easy limb bruising and menorrhagia, which required iron supplementation. She had a negative family history of abnormal bleeding. Initial laboratory studies included findings within reference intervals for complete blood cell count and activated partial thromboplastin time (aPTT) (30.8 s, reference interval 23–36 s), prolonged PT (20.3 s, reference interval 11.0–15.0 s), and International Normalized Ratio (INR) (1.78, reference interval 0.9–1.2). No preanalytical artifacts were identified, and the result of a repeat PT was also prolonged.

DISCUSSION

LABORATORY EVALUATION OF PROLONGED RESULTS FOR SCREENING COAGULATION TESTS

PT and aPTT are commonly requested screening tests. In vivo, the initiation of coagulation depends on tissue factor–mediated factor VII (FVII) activation, and sustained thrombin generation requires activation of factors XI, IX, VIII, X, and V. For the interpretation of PT and aPTT results, however, coagulation factor activation culminating in a fibrin clot can be organized into intrinsic, extrinsic, and common pathways (Fig. 1). An isolated result showing aPTT prolongation suggests a deficiency or inhibitor of one or more of the intrinsic pathway clotting factors (prekallikrein, high molecular weight kininogen, factors XII, XI, IX, and VIII). An isolated PT prolongation suggests a deficiency or inhibition of the extrinsic pathway (FVII), but mild factor X, V, and II deficiencies are also possible causes. Prolongation of both aPTT and PT suggests a deficiency or inhibition of the common pathway coagulation factors (factor X, V, and II), or a qualitative or quantitative fibrinogen defect.

When evaluating an unexpected prolonged aPTT and/or PT result, the first step is to rule out preanalytical causes of inaccuracy (1). Anticoagulant contamination due to blood collection from a venous or arterial line flushed with heparin is a common artifact, and although most commercial PT reagents contain a substance capable of neutralizing approximately 2 U/mL of heparin, this capacity can be overwhelmed if blood is collected from heparin-containing catheters. Other preanalytical variables include the use of collection tubes containing a higher sodium citrate anticoagulant concentration (3.8% instead of 3.2%), hemolyzed samples interfering with photo-optical clot detection methods, and a prolonged time lapse between specimen collection and performance of aPTT (>4 hours) and PT (>24 hours) assays. An increase of the citrate:plasma ratio, which decreases the ionized calcium concentration [e.g., in samples from patients with high hematocrit (>55%) or samples collected in underfilled collection tubes] may produce erroneously prolonged PT and aPTT results.

The second step in evaluating an unexpected prolonged aPTT and/or PT result should be to repeat the aPTT or PT assay, taking care to eliminate potential sources of preanalytical artifact. If the screening coagulation test continues to show prolonged times, the third step is to perform a mixing study on a 50:50 mixture of patient plasma and normal pooled plasma. Correction to within the reference interval is consistent with a deficiency of one or more factors, and no or partial correction is consistent with inhibitor activity due to an anticoagulant, a factor-specific neutralizing antibody, or a nonspecific lupus anticoagulant.

ADDITIONAL PATIENT DATA

We performed a mixing study, and the PT was corrected to 14.5 s, a result suggestive of an FVII deficiency because lupus anticoagulant, common pathway, and fibrinogen defects usually prolong the aPTT as well. FVII activity measurement performed on a mechanical clot detection instrument with rabbit thromboplastin activator was 5% (reference interval 60%–150%). Additional investigations ruled out a coexisting common pathway defect (factor V 144%, factor X 86%, factor II not done), fibrinogen deficiency (fibrinogen,
3700 mg/L, reference interval 1800–4000 mg/L), a direct thrombin inhibitor (thrombin time, 18.4 s, reference interval 16–22 s), and a nonspecific inhibitor (negative lupus anticoagulant screen).

The differential diagnosis for a patient with an isolated FVII deficiency is limited, because conditions that substantially reduce coagulation factor synthesis additionally prolong the aPTT. It is unlikely that subtle changes in hepatic function or vitamin K metabolism would produce such a profound, isolated decrease of FVII as was seen in this case. The patient described a varied diet with no alcohol consumption, and her medical records from 2 years earlier documented normal hepatic function, normal aPTT, and prolonged PT results. These findings support a diagnosis of FVII deficiency.

**DIAGNOSIS**

FVII Deficiency.

**OVERVIEW OF FVII DEFICIENCY**

Isolated FVII deficiency may be acquired or inherited. Reports of acquired FVII deficiency are rare, and the majority of cases are associated with malignancy, sepsis, and/or bone marrow transplantation (2). In some cases, in vitro evidence supports production of autoantibodies that either neutralize FVII activity or accelerate its clearance. A case of transient acquired FVII deficiency associated with surgery has been successfully treated with recombinant activated FVII (rFVIIa, NovoSeven) (2).

Congenital FVII deficiency has an estimated prevalence of 1:500 000 (3) and is often discovered incidentally. Patients may be asymptomatic or have spontaneous joint and cerebral hemorrhages. Bleeding complications usually occur in homozygotes and compound heterozygotes, and unlike factor VIII and IX deficiencies (found in males with hemophilia A and B, respectively), the degree of FVII deficiency is poorly correlated with bleeding tendency.

A public database (europium.csc.mrc.ac.uk) lists 136 unique mutations in the coagulation FVII (F7) gene, along with associated FVII activity, antigen concentration, and bleeding severity. Most F7 mutations are missense and occur in the catalytic domain, but various types of mutations have been identified at sites scattered throughout the F7 gene. Uncommon F7 mutations may cause life-threatening hemorrhages in neonates. Such mutations typically prevent protein expression and produce FVII activities <2%. F7 mutations associated with mild-to-moderate bleeding histories are usually missense mutations affecting circulating FVII, with activities ranging from 1% to 50%. Asymptomatic FVII-deficient
patients have FVII activities of 4%–61%, and in these cases all F7 mutations are missense.

In the presence of rabbit tissue factor, used in some commercial PT reagents, some F7 mutations show only negligible FVII activity but may show approximately 30% activity in the presence of human tissue factor (4). The first variant to display different FVII activities depending on the source of tissue factor was named FVII Padua (5). This variant is due to glutamine substitution for arginine at position 304 (R304Q), which impairs formation of the FVIIa–tissue factor–FX complex (6), resulting in phenotypes’ ranging from asymptomatic to moderate bleeding. To avoid reporting an inaccurately low activity, laboratories should always use recombinant human thromboplastin when evaluating patients with FVII deficiency.

PATIENT MANAGEMENT
Our patient presented with a prolonged PT and 5% FVII activity, findings that were not entirely consistent with her mild bleeding history. Therefore, we repeated the FVII activity assay with recombinant human thromboplastin and obtained an activity of 31%. In consultation with the patient’s orthopedic surgeon, who estimated intraoperative blood requirements of approximately 4–6 units due to the anticipated duration and complexity of the surgery, we recommended transfusion with 5 units of fresh frozen plasma during the operation, equivalent to approximately 20% of the patient’s plasma volume. Recombinant FVIIa was available if severe hemorrhage developed. The patient received 2 units of red cells intraoperatively, and the surgeon described normal hemostasis. Postoperatively, the surgery team suspected formation of a deep hematoma due to persistent anemia despite transfusion of 5 additional units of red cells over 6 days. However, the patient’s hemoglobin stabilized, wound drainage stopped, she received no additional blood components, and she was discharged 12 days postsurgery. At an outpatient visit 1 month later, the patient’s incision was healed without evidence of bleeding and her physical activity level was improving.

Fig. 2. Algorithm for managing FVII deficiency.
*Mild bleeding history includes minor epistaxis, menorrhagia, gastrointestinal bleeding, joint and soft tissue bleeding due to trauma, and/or bleeding during or after surgery. †Severe bleeding includes life-threatening mucosal or gastrointestinal bleeding, spontaneous soft tissue and joint bleeds, and/or central nervous system and ocular bleeding.
SUMMARY AND RECOMMENDATIONS

In the absence of a severe bleeding history, most FVII-deficient patients are at risk for hemorrhagic complications only following major surgery or trauma. Each case requires individual management because of the poor correlation between F7 mutations, FVII activity, and phenotype. The patient’s bleeding history, current clinical situation, and an FVII activity assay performed with recombinant human thromboplastin must guide initial management of FVII deficiency. FVII can be temporarily supplemented by infusing fresh frozen plasma, prothrombin complex concentrates containing factors X, IX, VII, and II derived from plasma, and recombinant FVIIa (NovoSeven®). Fresh frozen plasma infusion may produce volume overload in susceptible patients, and thrombotic complications are a potential risk with prothrombin complex concentrates and NovoSeven® therapy.

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**References**


**Commentary**

Sandra C. Hollensead

Understanding of thrombin formation in vivo has been greatly enhanced by the development of a cell-based model of coagulation. In this model, all clotting is initiated by the combination of tissue factor and activated coagulation factor VII (FVIIa). The source of the tissue factor may be extravascular or intravascular. Once initial thrombin is formed, activated coagulation factors VIII and IX greatly accelerate the process, leading to a “thrombin burst.” This mechanism explains why deficiencies of factors VIII and IX lead to problematic clinical bleeding, whereas deficiencies of factors VII or XI may not.

The cell-based model of coagulation provides a good underlying theory supporting the therapeutic use of recombinant FVIIa. The use of this product is often guided by protocols and measurement of FVII activity levels. Thus the case report by Hood and Eby is timely, because requests for measurement of FVII activity are increasing with increasing use of recombinant FVIIa. The reported case emphasizes the use of recombinant human thromboplastin prothrombin time...
reagents in obtaining the best measurement of FVII activity. The algorithm reflects the need to tailor infusions of recombinant FVIIa to patient condition. Patients with congenital FVII deficiency and no history of bleeding will not need the amount of factor that a patient with coumadin-related intracerebral hemorrhage requires.

Good medical theory and knowledge leads to good medical diagnosis and treatment. Enhanced understanding of coagulation allows the development of therapies targeted to specific coagulation defects uncovered in the laboratory. Although laboratorians still must master the models of intrinsic and extrinsic clotting as a means to interpret laboratory tests, understanding gained from the new cell-based model of coagulation is indeed a welcome change.

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Commentary
Elizabeth M. Van Cott

This case provides useful information on the laboratory evaluation of a prolonged prothrombin time (PT). The great majority of PT prolongations are due to acquired rather than hereditary causes, but diagnosis of hereditary deficiencies when they are present is important for appropriate patient care. Common acquired causes of PT prolongations include warfarin therapy, vitamin K deficiency, decreased hepatic synthesis of coagulation factors, and disseminated intravascular coagulation. In each of these scenarios for acquired PT prolongation, multiple factors are typically deficient. Although mixing studies are useful in the evaluation of a prolonged PT, when multiple factor deficiencies are present the mixing study does not always correct completely into the reference interval. Mixing studies perform more reliably when only a single factor deficiency is present. The reason for the incomplete correction is presumably because, in the resulting mix, multiple factors are present in amounts at the lower end of the reference interval, a situation that can lead to a PT prolongation even though none of the factors are deficient.

It is also important to keep in mind the lack of utility of heparinase in the evaluation of a prolonged PT. Heparinase is useful in the evaluation of a prolonged activated partial thromboplastin time (PTT), because if the PTT is normal after treating the specimen with heparinase, then the PTT prolongation can be attributed to heparin. Thus, it is tempting to use heparinase to determine if heparin is also the cause of a prolonged PT. As noted by Hood and Eby, high amounts of heparin can overwhelm the heparin-neutralizing capability of PT reagents, resulting in a PT prolongation. However, in my experience, heparinase is not useful for evaluating prolonged PTs, because even when it is known that the PT prolongation is due to heparin, the heparinase is unable to correct the PT to reference interval values, possibly because of the increase in tissue factor pathway inhibitor that occurs with heparin therapy.

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References