

Bleeding represents a frequent complication of warfarin therapy. The risk of bleeding increases as the prothrombin time becomes more prolonged. However, bleeding episodes often occur when patients are in "therapeutic range" of the prothrombin time. Some of the variability between the risk of bleeding and the prothrombin time can be ascribed to differing sensitivities of the reagents and instrumentation used. The major difficulty with the prothrombin time is that the kinetic screening test itself represents the net effect of several enzymatic reactions and a final nonenzymatic (polymerization) step. The three different vitamin K-dependent clotting factors involved in the prothrombin time have half-lives that vary from 6 to 60 h and concentrations in plasma that range from 10 nM for factor VII to 1.4 mM for prothrombin (1). Their relative rates of synthesis and degradation appear to differ in vivo; these variables all contribute to the lack of sensitivity of the prothrombin time in reflecting a person's risk of bleeding on warfarin. A much stronger association of bleeding episodes is with low levels of "normal" prothrombin antigen, i.e., immunoreactive prothrombin using antibodies specific for the calcium-dependent conformation of the gamma-carboxyglutamic or Gla domain (2). Nevertheless, the prothrombin time remains the screening test used to monitor warfarin therapy and distinguish patients at inordinate risk of bleeding.

In persons on chronic warfarin therapy, there is usually a similar degree of prolongation of the prothrombin and partial thromboplastin times. When differences in procoagulant levels of different vitamin K-dependent factors are observed in persons chronically on warfarin, they may reflect either a non-steady state and/or differences in baseline levels. If a patient happens to have a high-normal baseline factor VII level and a low-normal factor IX, warfarin would exert a greater effect on the latter. For most patients considered to be in the therapeutic range, levels of the four vitamin K-dependent factors are similar and around 10–20% of normal.

Chu et al. (3) describe a patient on warfarin who had a bleeding complication while his prothrombin time was in the therapeutic range. Curiously, the partial thromboplastin time appeared disproportionately prolonged. On specific factor assays, the factor IX clotting activity was < 1% of normal when the prothrombin time was in "therapeutic range." This suggested that his factor IX had increased sensitivity to warfarin and that the prothrombin time was a particularly poor indicator of his anticoagulant effect. A mutation predicting a substitution of an Ala to Thr (at the –10 position in the propeptide region) was found. Ala is highly conserved at the comparable position of vitamin K-dependent proteins and is likely important in binding of carboxylase for gamma-carboxylation. A 59-amino acid polypeptide containing the factor IX propeptide (with either Thr of Gly substituting Ala at the –10 position) and the Gla domain was prepared by site-directed mutagenesis, expressed and purified. The K_m app was over 30-fold

higher for the mutant peptides indicating a significantly reduced binding affinity for carboxylase. Of interest, while not on warfarin, this patient had a normal factor IX clotting activity (148%, 120%). Thus the mutation represents a variant that predisposed him to warfarin toxicity.

Identified mutations in genes usually are associated with clinical disorders due to decreased function of a given gene or its protein or with asymptomatic variants or polymorphisms. The case study by Chu et al. (3) underscores that erstwhile asymptomatic variants may predispose a person to complications from an environmental change such as a common anticoagulant drug. A second important message from the evaluation of this patient is that by pursuing inconsistencies in laboratory test results, clinicians can understand "atypical" responses to therapy. Indeed, the combination of a partial thromboplastin time that was disproportionately prolonged compared to the prothrombin time and a factor IX clotting activity level that was lower than levels of other vitamin K-dependent blood clotting factors suggests increased sensitivity of a person's factor IX to warfarin. A mutation in the putative carboxylase binding domain in the propeptide region of factor IX was found. This provides a likely explanation of the increased sensitivity of this patient's factor IX to warfarin. Although there are some limitations and assumptions associated with in vitro studies of peptides and use of systems from different species (i.e., bovine microsomes for carboxylase), expression of mutant peptides and demonstration of reduced binding to carboxylase provide strong evidence to support the hypothesis.

Examples such as the patient described by Chu et al. (3) specify redefinition of asymptomatic variants. Furthermore, they remind us of potential interactions of genetic predispositions and environmental risks and demonstrate the ability of molecular biology to re-create mutations and thus approach a formal proof that a given patient's mutation is responsible for an observed clinical outcome.

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References

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