Thrombosis Research 132 (2013) e70–e75

Contents lists available at SciVerse ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

Contributions of procoagulants and anticoagulants to the international normalized ratio and thrombin generation assay in patients treated with warfarin: Potential role of protein Z as a powerful determinant of coagulation assays

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ARTICLE INFO

Article history:
Received 12 January 2013
Received in revised form 20 May 2013
Accepted 21 May 2013
Available online 13 June 2013

ABSTRACT

Background: The effects of warfarin are measured with the international normalized ratio (INR). However, the thrombin generation assay (TGA) may offer more information about global coagulation. We analyzed the monitoring performance of the TGA and INR and investigated the impact of procoagulants (fibrinogen, factor (F)II, FVII, FIX, and FX) and anticoagulants (proteins C, S, and Z) on them.
Methods: The TGA was performed on a calibrated automated thrombogram, producing lag time, endogenous thrombin potential (ETP), and peak thrombin in 239 patients treated with warfarin. Pro- and anticoagulant levels were also measured.
Results: The INR was significantly and inversely correlated with ETP. The therapeutic range of ETP comparable to an INR range of 2.0–3.0 was 290.1–494.6. ETP showed comparable performance to the INR as a warfarin-monitoring parameter with respect to clinical complication rate. The median levels of FII, FVII, FIX, and FX and proteins C and Z tended to decrease gradually with increasing anticoagulation intensity according to the INR or ETP. Of note, protein Z levels decreased dramatically with increasing anticoagulation status. INRs were significantly determined by FII, FVII, and protein Z. ETP was significantly dependent on FVII, and proteins C and Z concentration. Protein Z significantly reduced the total amount of thrombin generation and prolonged PT value *in vitro*.
Conclusions: The INR and ETP exhibit similar efficacy for warfarin monitoring according to the clinical complication rate. Protein Z is considered to be a significant determinant of INR and ETP in patients on warfarin therapy.

Introduction

Warfarin anticoagulation is widely used to prevent thromboembolism in prosthetic heart valves, atrial fibrillation, and ischemic heart disease [1]. Warfarin inhibits vitamin K reductase and then blocks the regeneration of the active form of vitamin K. Vitamin K is necessary for the formation of γ-carboxyglutamic acid in a series of amino acid residues within vitamin K-dependent proteins such as coagulation factor (F) II, FVII, FIX, FX as well as proteins C, S, and Z. Warfarin therapy requires close monitoring of the prothrombin time (PT)/international normalized ratio (INR) to reduce bleeding or thrombotic risk. However, the INR has several drawbacks. The international sensitivity index assignment of local thromboplastin reagent/test system is still not consistent among clinical laboratories [2]. Moreover, it is doubtful that the INR accurately reflects the overall in vivo hemostatic capacity. FII and FX levels play more important roles in the antithrombotic effect than FVII or FIX [1]. However, it is still unclear whether the INR accurately reflects FII and FX levels. Although warfarin inhibits the synthesis of anticoagulants including proteins C and S as well as procoagulants, it is unknown whether anticoagulants levels affect INR.

Recently, increasing evidence suggests that the identification of thrombin generation provides useful information about global hemostatic capacity [3]. In the thrombin generation assay (TGA), which uses an automated calibrated thrombogram, thrombin generation curves can be operationally characterized as exhibiting initiation, propagation, and termination phases. After stimulation with tissue factor (TF), the resultant formation of endogenous thrombin potential (ETP) is measured in plasma. ETP has been identified as a good

Abbreviations: F, coagulation factor; PT, prothrombin time; INR, international normalized ratio; TGA, thrombin generation assay; TF, tissue factor; ETP, endogenous thrombin potential.

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http://dx.doi.org/10.1016/j.thromres.2013.05.015

Table 1
Correlations among global coagulation tests and proteins (n = 239).

	INR	ETP	Fibrinogen	FII	FVII	FIX	FX	Protein C	Protein S
ETP	−0.769	1							
Fibrinogen	−0.023	−0.018	1						
FII	−0.419[b]	0.213[b]	0.133[a]	1					
FVII	−0.472[b]	0.255[b]	0.110[a]	0.807[b]	1				
FIX	−0.382[b]	0.218[b]	0.252[b]	0.740[b]	0.770[b]	1			
FX	−0.390[b]	0.191[b]	0.154[b]	0.864[b]	0.758[b]	0.677[b]	1		
Protein C	−0.434[b]	0.196[b]	0.091	0.873[b]	0.883[b]	0.736[b]	0.796[b]	1	
Protein S	−0.260[b]	0.121	0.048	0.593[b]	0.507[b]	0.565[b]	0.639[b]	0.538[b]	1
Protein Z	−0.588[b]	0.515[b]	0.010	0.281[b]	0.258[b]	0.259[b]	0.271[b]	0.241[b]	0.231[b]

Abbreviations: ETP, endogenous thrombin potential; F, factor; INR, international normalized ratio.
[a] $P < 0.05$; [b] $P < 0.001$.

marker of prothrombotic and hemorrhagic diseases [3,4]. ETP may serve as a more sensitive parameter for laboratory monitoring of warfarin. A recent study reports that the INR and ETP are well correlated and that thrombin generation varies widely in individuals with the same INR—the cause of which remains unclear [5].

Protein Z is a vitamin K-dependent glycoprotein that serves as a cofactor for the inhibition of factor Xa by protein Z-dependent protease inhibitor [6]. Decreased plasma protein Z levels are reported in patients on warfarin therapy, newborns, and those with liver disease [6,7]. Notably, in chronic warfarin therapy, protein Z plasma levels are more profoundly decreased than those of protein C or the other vitamin K-dependent proteins [7]. Therefore, warfarin-induced protein Z deficiency might influence the measurement of the INR. To our knowledge, no study has investigated the actual effect of protein Z on INR or thrombin generation.

This study compared the therapeutic monitoring performance of ETP with that of the INR with respect to clinical complication rates. In addition, the influences of procoagulants (fibrinogen, FII, FVII, FIX, and FX) and anticoagulants (proteins C, S, and Z) on the INR and TGA in patients treated with warfarin were investigated.

Materials and Methods

Study Population

A total of 239 patients (129 men and 110 women; mean age, 61 years; age range, 26–89) taking oral anticoagulants for more than 6 weeks were recruited for this study. The patients were receiving warfarin therapy to prevent or treat various clinical conditions including prosthetic heart valves (n = 123), atrial fibrillation (n = 80), ischemic heart failure (n = 18), and deep vein thrombosis (n = 18). The subjects' INRs ranged from 1.0 to 4.26. Exclusion criteria were age < 19 years and antithrombotic therapy other than warfarin or anti-platelet agents. Demographic and clinical data including bleeding and thrombotic events were obtained from medical records. This study was approved by the Institutional Review Board of Seoul National University Hospital.

Blood Samples and Assays

Peripheral blood was collected in commercially available vacutainer (3.2% sodium citrate tube; Becton Dickinson, San Jose, CA, USA). Whole blood was centrifuged for 15 minutes at 1550 ×g at room temperature, and the tests were performed within 3 hours of blood sampling.

PT was assayed according to the standard one-stage clotting assay on a STA-R analyzer (Diagnostica Stago, Asnières, France). Plasma fibrinogen was measured using the Fibrinogen-C XL kit (Instrumentation Laboratory, Lexington, MA, USA) according to the Clauss method. FII, FVII, and FX levels were determined by a one-stage PT-based clotting assay triggered with RecombiPlasTin (Instrumentation Laboratory); FIX levels were measured by a one-stage activated partial thromboplastic time (aPTT)-based clotting assay triggered with SynthASil thromboplastin (Instrumentation Laboratory). The factor assays used commercially prepared plasmas deficient in one of these factors, i.e., <1% of the deficient factor but adequate concentrations of the other factors that influence the PT and aPTT. All coagulation factor assays were performed on an ACL Top (Beckman Coulter, Fullerton, CA, USA).

Protein C activity was measured by functional clotting protein C assay on the basis of the prolongation of the aPTT in the presence of activated protein C using a ProClot kit (Instrumentation Laboratory). Free protein

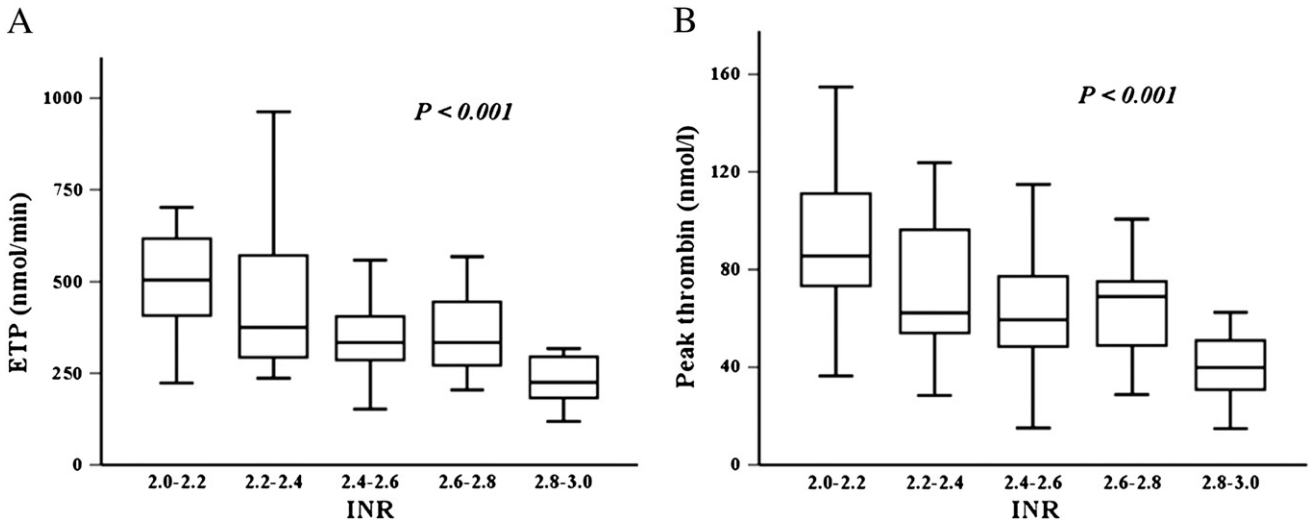

Fig. 1. Inter-individual variation of (A) endogenous thrombin potential (ETP) and (B) peak thrombin at the narrow ranges of the international normalized ratio (INR).