International Normalized Ratio Versus Plasma Levels of Coagulation Factors in Patients on Vitamin K Antagonist Therapy

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• ***Context.*—The key question when managing patients on warfarin therapy who present with life-threatening bleeding is how to use the international normalized ratio (INR) to best direct corrective therapy. The corollary question for the clinical laboratory is at what level will the INR reflect a critical value that requires notifying the clinician.**

***Objective.*—To determine the levels of vitamin K–dependent factors over a range of INR values.**

***Design.*—Evaluation of the vitamin K–dependent coagulation factor levels on plasma remnants from patients in whom a prothrombin time and INR was ordered to monitor warfarin therapy. There were a total of 83 specimens evaluated with an INR range from 1.0 to 8.26.**

***Results.*—The mean activity levels of all 4 factors remained near or above 50% when the INR was less than 1.5. The average factor X level was 23% when the INR range was 1.6 to 2.5, but levels of factors II, VII, and IX did not drop below the hemostatic range until the INR was greater than 2.5. At an INR of 3.6 or more, the activity levels of all 4 factors were less than 30% in more than 90% of the specimens.**

***Conclusion.*—Levels of factors II, VII, IX, and X declined with increasing INR but not at the same rate and not to the same level at a given INR. However, most of the values were below the hemostatic value once the INR was 3.6 or more, the level that was also considered critical for physician notification.**

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Vitamin K antagonist (VKA) therapy is routinely monitored by the international normalized ratio (INR), determined from the prothrombin time (PT) test result by a standard formula:

$$\mathrm{INR} = \left(\frac{\text{PT of the patient}}{\text{geometric mean of the PT reference range}}\right)^{\text{ISI of the reagent}},$$

where ISI is the international sensitivity index. An INR value in the range of 2.0 to 3.5 is generally considered therapeutic. The evidence-based clinical practice guidelines for antithrombotic and thrombolytic therapy published by the American College of Chest Physicians form the basis of management of VKA therapy as well as management of supratherapeutic INRs.[1] The recommendation for management of an INR that is greater than the upper limit of the therapeutic range understandably varies more so on whether the patient is bleeding and on the nature and degree of bleeding than on the magnitude of the elevation of the INR alone. However, 2 questions frequently raised by clinicians and laboratory professionals in their daily practice are (1) what does the INR value reflect in the levels of coagulation factors affected by the VKA therapy and (2) what value of INR should be considered a critical level requiring immediate notification to the physician responsible for patient management. Although some work has been done to determine the coagulation factor levels in specimens from patients on VKA therapy, the available information does not directly answer these questions. We therefore studied the relationship between various INR levels and the levels of individual coagulation factors affected by VKA therapy from plasma sample remnants of patients in whom a PT/INR was ordered to monitor warfarin therapy.

MATERIALS AND METHODS

Platelet-poor plasma remnants of 83 citrated blood specimens were collected from 75 patients (equally distributed between men and women and in the age range of 31 to 93 years with a median of 68 years) who had a physician order for the PT/INR test for monitoring of VKA therapy. These specimens were selected to represent an INR range of 1 to 8. All blood specimens were collected in blue-top evacuated tubes containing 3.2% sodium citrate (Greiner Bio-One Vacuette North America, Inc, Monroe, North Carolina). The blood to anticoagulant ratio in each tube was 9:1. The PT/INR was performed on the day of specimen collection on a mechanical analyzer (STAR-evolution or STAR, Diagnostica Stago, Inc, Parsippany, New Jersey) using a thromboplastin reagent with an ISI of 1.24 (Neoplastin C+, Diagnostica Stago). Aliquots were frozen at −70°C within half an hour of performing the PT/INR and kept at that temperature until the time the factor assays could be performed. Factor assays

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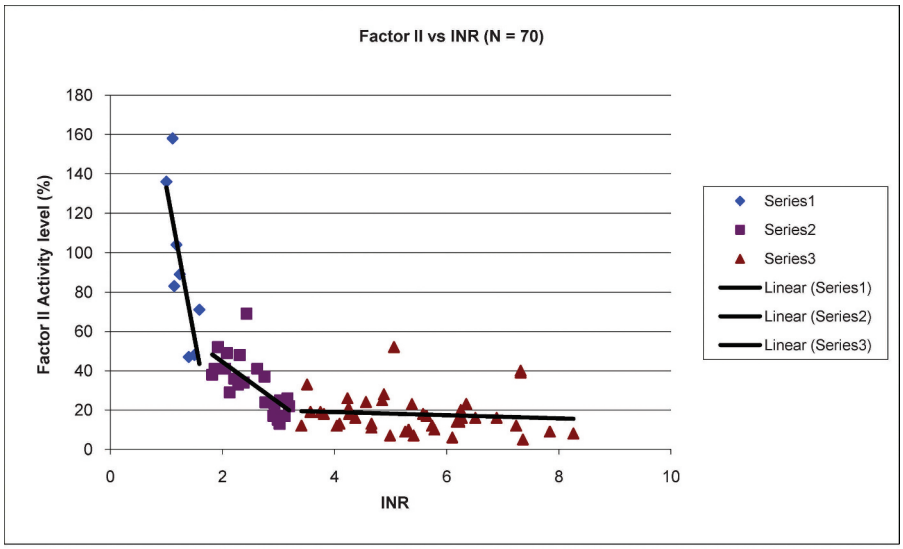

Figure 1. *Plasma factor II activity levels versus international normalized ratio (INR).*

Series 1: INR range 1.0 to 1.6 (N = 8)
Series 2: INR range 1.8 to 3.2 (N = 22)
Series 3: INR range 3.4 to 8.3 (N = 40)

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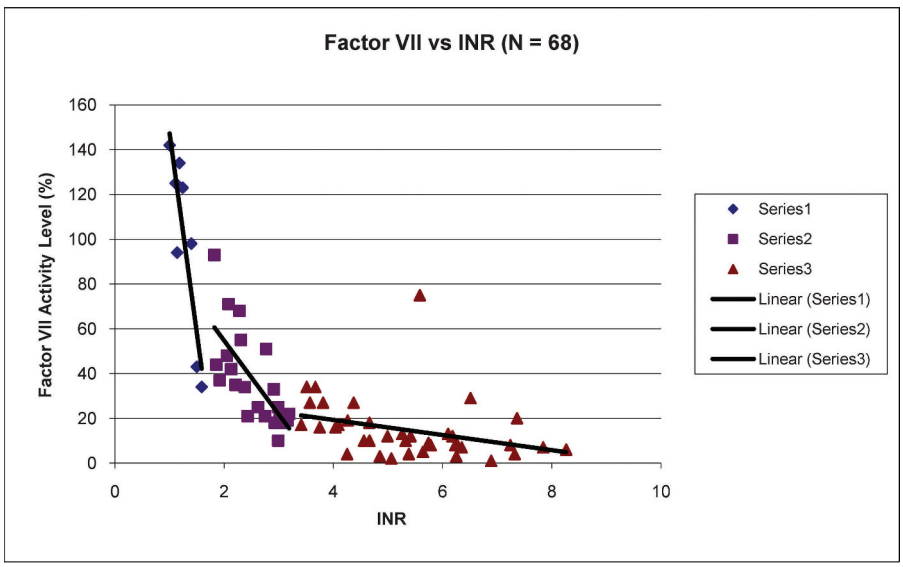

Figure 2. *Plasma factor VII activity levels versus international normalized ratio (INR).*

Series 1: INR range 1.0 to 1.6 (N = 8)
Series 2: INR range 1.8 to 3.2 (N = 22)
Series 3: INR range 3.4 to 8.3 (N = 38)

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were performed within 5 months from the date of freezing the aliquots. The frozen specimens were thawed at 37°C only once and activity levels of coagulation factors II, V, VII, IX, and X were measured on a photo-optical analyzer (ACL [Automated Coagulation Laboratory] Advance, Instrumentation Laboratory Company, Inc, Lexington, Massachusetts), using PT-Fibrinogen HS (high sensitivity) reagent for factors II, V, VII, and X and APTT-SP reagent for factor IX (Instrumentation Laboratory). The factor-deficient substrates (immunodepleted) were obtained initially (for one third of the specimens) from Instrumentation Laboratory and later (for the remaining two thirds of the specimens) from Precision Biologic, Inc, Dartmouth, Nova Scotia, Canada. Substrates from the 2 sources were comparable (correlation coefficients of 0.972 or greater) as judged by the correlation studies performed at the time of the switch, which coincided with the lot and/or reagent change made annually in our laboratory. Specimens in which the factor V activity level was less than 50% (11 of the original 83 specimens) were presumed to be from patients with liver disease and hence excluded from the data analysis. Consequently, a total of 72 specimens were included in the final evaluation. The percent activity levels of each factor were plotted against the INR values and best-fit lines were generated using Excel software (Microsoft, Redmond, Washington).

RESULTS

The activity levels of each of the 4 vitamin K–dependent zymogens (II, VII, IX, and X) decreased as the INR level increased. Individual factor activity levels are plotted against the INR values in Figures 1 through 4. The curvilinear nature of the plotted data and the apparent triphasic pattern of decline in individual factor activity levels necessitated the use of segmented linear regression

Distribution of Plasma Factor Activity Levels of Specimens With International Normalized Ratio (INR) 3.6 or More					
Factor	No. of Specimens With INR 3.6 or More	Specimens With <10% Activity, No. (%)	Specimens With 10% to 20% Activity, No. (%)	Specimens With 21% to 30% Activity, No. (%)	Specimens With >30% Activity, No. (%)
II	38	7 (18.4)	21 (55.3)	7 (18.4)	3 (7.9)
VII	36	16 (44.4)	14 (38.9)	4 (11.1)	2 (5.6)
IX	35	2 (5.7)	20 (57.1)	10 (28.6)	3 (8.6)
X	39	22 (56.4)	15 (38.5)	2 (5.1)	0 (0)

to generate the best-fit lines. The segmentation was based on visual inspection of the curves for all 4 factors. The 3 segments listed as series 1, series 2, and series 3 in Figures 1 through 4 are represented by the respective INR ranges of 1.0 to 1.6, 1.8 to 3.2, and 3.4 to 8.3 for each factor.

Factor II

The results of factor II activity level measurements performed on 70 of the total 72 specimens are plotted in Figure 1. There was not sufficient plasma to perform measurement in 2 of the specimens. When the INR was 1.6 to 2.0, the average factor II activity level was 50%. An INR in the range of 2.1 to 3.0 yielded an average factor II activity level of 33%. When the INR was 3.1 to 3.5, the average factor II activity level was 22%. Of the 38 specimens with an INR value of 3.6 or greater, 3 (7.9%) had greater than 30% activity, 7 (18.4%) had 21% to 30% activity, 21 (55.3%) had 10% to 20% activity, and 7 (18.4%) had less than 10% activity (Table).

Factor VII

The results of factor VII activity level measurements performed on 68 of the total 72 specimens are plotted in Figure 2. There was not sufficient plasma to perform measurement in 4 of the specimens. When the INR was 1.6 to 2.5, the average factor VII activity level was 50%. When the INR was 2.6 to 3.5, the average factor VII activity level was 24%. Of the 36 specimens with an INR value of 3.6 or greater, 2 (5.6%) had greater than 30% activity, 4 (11.1%) had 21% to 30% activity, 14 (38.9%) had 10% to 20% activity, and 16 (44.4%) had less than 10% activity (Table).

Factor IX

The results of factor IX activity level measurements performed on 60 of the total 72 specimens are plotted in Figure 3. There was not sufficient plasma to perform measurement in 12 of the specimens. The factor IX activity level averaged 63% when the INR was in the range of 1.5 to 2.0. It dropped to an average level of 43% when the INR was 2.1 to 2.5. At an INR between 2.6 and 3.5, the average factor IX activity level was 32%. Of the 35 specimens with an INR value of 3.6 or greater, 3 (8.6%) had greater than 30% activity, 10 (28.6%) had 21% to 30% activity, 20 (57.1%) had 10% to 20% activity, and 2 (5.7%) had less than 10% activity (Table).

Factor X

The results of factor X activity level measurements performed on 71 of the total 72 specimens are plotted in Figure 4. There was not sufficient plasma to perform measurement in 1 of the specimens. When the INR was in the range of 1.6 to 2.5, the average factor X activity level was 23%. At an INR between 2.6 and 3.5, the average factor X activity level was 13%. Of the 39 specimens with an INR value of 3.6 or greater, none had greater than 30% activity, 2 (5.1%) had 21% to 30% activity, 15 (38.5%) had 10% to 20% activity, and 22 (56.4%) had less than 10% activity (Table).

A side-by-side comparison of mean activity levels of all 4 factors against corresponding INR values is illustrated in Figure 5. The decline in activity levels of all 4 factors appeared to follow a triphasic pattern. An initial sharp drop with an increase in INR from 1.0 to 1.6 was followed by a gradual decline with INR in the range of 1.8 to 3.2, ultimately reaching what can be considered a plateau when the INR was 3.6 or more.

COMMENT

Coumadin (crystalline warfarin sodium) is the most commonly used VKA for the prophylaxis and/or treatment of arterial and venous thromboembolism. It interferes with the gamma carboxylation of the vitamin K–dependent factors, which include coagulation factors II, VII, IX, and X, and the anticoagulant proteins C and S. This study was limited to the measurement of the activity levels of the vitamin K–dependent coagulation factors from plasma remnants of specimens sent to the clinical laboratory for determination of PT/INR.

As expected, the levels of all 4 vitamin K–dependent coagulation factors, II, VII, IX, and X, declined with increasing INR. Although the pattern of decline was similar among all 4 factors, the rate and magnitude was greatest for the factor X activity (Figure 5). These findings suggest that the vitamin K–dependent factor levels do not decrease at the same rate and magnitude in response to VKA therapy.

The minimum hemostatic levels of the vitamin K–dependent factors have been reported to be in the ranges of 20% to 40% for factor II, 25% to 30% for factor IX, and 10% to 20% each for factors VII and X.[2] However, when a patient is on VKA therapy, the determination of the critical value[3] as well as management decisions[1] are based on the INR. At an INR of 3.6 or greater, we observed the activity level of less than 10% in (1) 56.4% of specimens for factor X, (2) 44.4% of specimens for factor VII, (3) 18.4% of specimens for factor II, and (4) 5.7% of specimens for factor IX. In addition, there were occasional specimens with the activity levels of vitamin K–dependent coagulation factors that when associated with an inherited deficiency would be expected to be associated with significant bleeding. We observed a factor VII activity level of 1% at an INR of 6.89, a factor X activity level of 4% with an INR of 7.36 in 1 case and 8.26 in another case, a factor II activity level of 5% with an INR of 7.36, and a factor IX activity level of 8% with an INR of 6.51.

According to a survey conducted in 2000 by the Coagulation Resource Committee of the College of American Pathologists, the level of INR used as a critical value by the participant laboratories ranged between 4.0 and 6.0.[4] The most frequently chosen INR for the critical value was 5.0 (27%), followed by 4.0 (16%). An INR of 6.0 was chosen by 12% of laboratories. The reason for

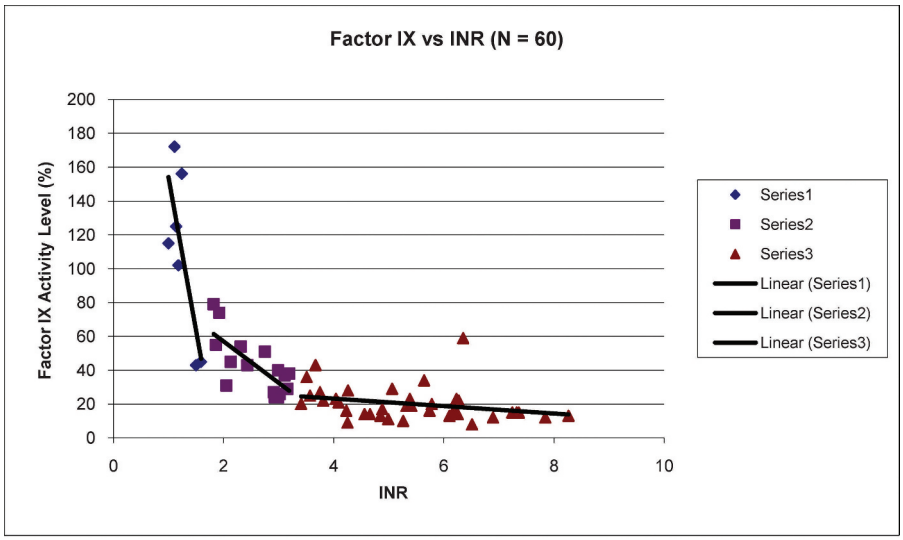

Figure 3. *Plasma factor IX activity levels versus international normalized ratio (INR).*

3 Series 1: INR range 1.0 to 1.6 (N = 7)
Series 2: INR range 1.8 to 3.2 (N = 16)
Series 3: INR range 3.4 to 8.3 (N = 37)

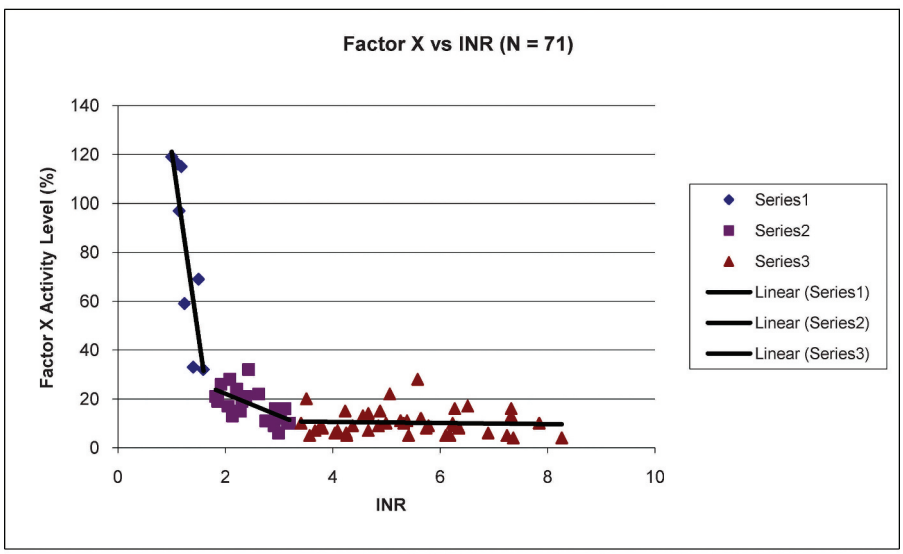

Figure 4. *Plasma factor X activity levels versus international normalized ratio (INR).*

4 Series 1: INR range 1.0 to 1.6 (N = 8)
Series 2: INR range 1.8 to 3.2 (N = 22)
Series 3: INR range 3.4 to 8.3 (N = 41)

choosing an INR of 5.0 for the critical value by most laboratories is believed to be the recommendations by the American College of Chest Physicians Conference on Antithrombotic and Thrombolytic Therapy for therapeutic intervention in patients on oral anticoagulant therapy when the INR is 5.0 or more. The survey, however, revealed the lack of consensus among the laboratories in determining and/or choosing the INR critical value. Our data suggest that when the INR is greater than 3.5, a significant number of patients may have factor activity far below the hemostatic level and therefore an INR of greater than 3.5 should be considered a critical value for the purpose of notification to the caregiver.

Previous studies evaluating the vitamin K–dependent factor levels in patients on VKA therapy focused on the evaluation of therapeutic levels rather than bleeding risk. Nonetheless, there are important similarities in the findings. Our study showed that although patterns of decline are similar, the rate and magnitude of decline may not be the same for all factors at a given INR; this is in agreement with previous reports.[5–7] Our observation of an apparent plateau effect at INR values greater than 3.6 is akin to the findings of Sarode et al,[8] who reported poor correlation between supratherapeutic INR (>5.0) and the levels of vitamin K–dependent coagulation factors. Also, of clinical importance is the confirmation that the factor X

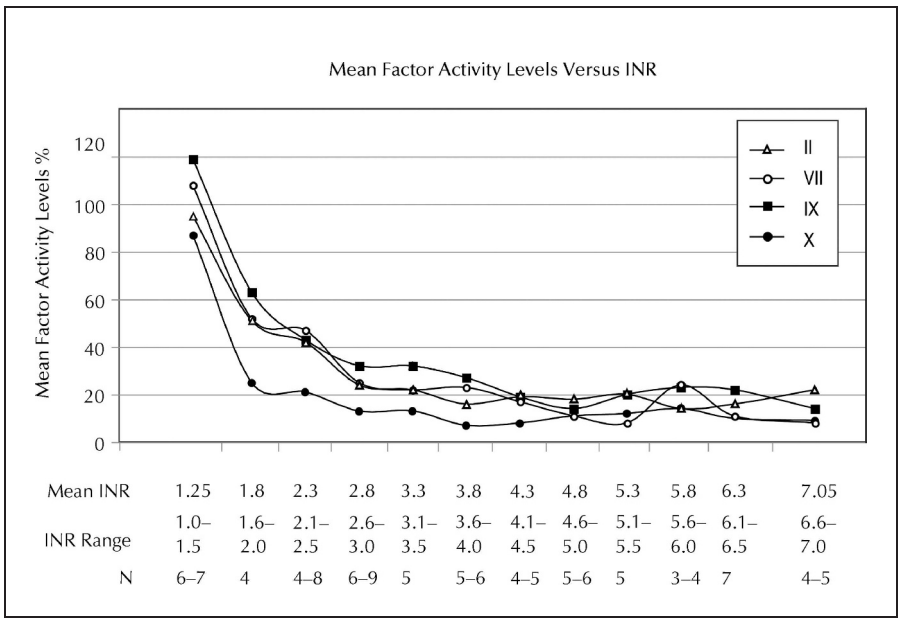

Figure 5. *A side-by-side comparison of mean factor activity levels versus mean international normalized ratio (INR) values. Each plotted point represents the mean of 3 or more data points as indicated on the x-axis.*

activity level is lower as compared with the levels of factors II, VII, and IX at the same INR value.[7]

These findings suggest that selection of appropriate therapy should be based on factor activity level(s) or their derivation from the INR result. When a patient presents with an elevated PT, it must first be established whether the coagulopathy is secondary to VKA therapy. If so, the INR, a mathematical derivative of the PT, becomes the laboratory value used, with or without the benefit of factor activity level measurements, to determine management of the patient. Treatment modalities for the actively bleeding patient who has been on VKA therapy include a recombinant VIIa product, fresh frozen plasma, and prothrombin complex concentrate.[3] The prothrombin complex concentrates contain factors, II, VII, IX, and X; optimal dosing of this product could be calculated using either measured or derived levels of these vitamin K–dependent factors.

Ideally, information from prospective evaluation of patients on VKA therapy presenting either for emergent surgery or with bleeding complications will contribute important information on clinical bleeding and hemostatic levels of these coagulation factors. The feasibility or practicality of measuring factor activity levels to guide therapeutic intervention in patients on VKA therapy should be further investigated in prospective studies.

CONCLUSION

The plasma activity levels of all 4 vitamin K–dependent coagulation factors (II, VII, IX, and X) remained above the reported respective minimum hemostatic levels when the INR was less than 1.5. Once the INR was in the range of 1.6 to 2.5, the mean factor X activity was 23%, a level considered near or at the minimum hemostatic level while the mean activity levels of factors II, VII, and IX remained above the minimum hemostatic levels at 35% to 45%. The average activity levels of factors II, VII, and IX approached the respective minimum hemostatic levels only when the INR was more than 2.5. Once the INR was equal to or greater than 3.6, most specimens had levels of these 4 factors that were less than 30%. Because an INR of 3.6 or more correlated with a drop in the 4 coagulation factors to below their respective minimum hemostatic levels in a significant number of the specimens, we propose that an INR value 3.6 or more be considered critical for the purpose of notification to the caregiver.

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