Comparison of a chromogenic factor X assay with international normalized ratio for monitoring oral anticoagulation therapy

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The purpose of the present study was to compare the international normalized ratio with a chromogenic factor X (CFX) assay for monitoring patients on oral anticoagulant therapy using the DiaPharma CFX method on a STA-R Evolution coagulation analyzer. International normalized ratio values were correlated with the CFX for determining normal, subtherapeutic, therapeutic and supratherapeutic ranges for these patients. Specimens were analyzed and grouped as normal or patients on oral anticoagulant therapy with international normalized ratios of less than 2.0, 2.0–3.0, and more than 3.0. Three hundred and nine randomly selected oral anticoagulant therapy patients were tested. The range of international normalized ratio and CFX in oral anticoagulant therapy patients was 0.92–12.76 and 9–132%, respectively. CFX was inversely related to international normalized ratio; $R = 0.964$ ($P < 0.0001$) (CFX = 13.2 + (5.3/international normalized ratio) + (81.3/international normalized ratio^2). Results by group were as follows: normal ($n = 30$), CFX range 72–131%, mean CFX 96%; international normalized ratio less than 2.0 ($n = 70$), CFX range 32–132%, mean CFX 53%; international normalized ratio 2.0–3.0 ($n = 135$), CFX range 18–48%, mean CFX 28%; international normalized ratio more than 3.0 ($n = 104$), CFX range 9–46%, mean CFX 21%. Sensitivity and specificity crossed at a CFX of 35.5%, which yielded a sensitivity of 91.7% and a specificity of 91.9% for discriminating international normalized ratio of at least 2.0. Area under the curve on receiver–operator curve using international normalized ratio was 0.984 ($P < 0.001$). In this randomly selected group of oral anticoagulant therapy patients and normal individuals at varying levels of anticoagulation, CFX correlated well with international normalized ratio as determined by $R = 0.964$. The data suggests that the CFX can be a useful tool for monitoring oral anticoagulation in patient populations in which confounders to international normalized ratio may be present. Further investigation with the use of CFX for monitoring is warranted in large patient populations on oral anticoagulant therapy, including follow-up for clinical outcomes. *Blood Coagul Fibrinolysis* **19:513–517 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

The international normalized ratio (INR) is the primary method for monitoring patients on oral anticoagulation therapy (OAT). However, INR values may be affected by the presence of lupus anticoagulant and other clinical and preanalytical variables [1,2]. This is especially true when the international sensitivity indices (ISI) of the thromboplastins have not been locally calibrated with the specific instrument combinations used in the testing facility [3–5].

In contrast, chromogenic factor X (CFX) assays have been shown to be insensitive to many of the variables that affect the INR [6,7], including lupus anticoagulant. Laboratories should not use the clottable factor X method that is phospholipid dependent to monitor patients with the presence of a lupus anticoagulant. Thromboplastin reagents used for the clottable factor X assay may not be suitable unless they are locally calibrated for the international sensitivity indices with each laboratory instrument/reagent combination. Thus, they may not be suitable for monitoring patients with evidence of a lupus anticoagulant [8–10]. Although clottable factor X and CFX have been reported useful for monitoring anticoagulation in patients receiving direct thrombin inhibitors such as argatroban [8–17], the range of therapeutic CFX values have not been defined in patients receiving warfarin therapy. The objectives of the present study were two-fold: to assess the relationship between CFX and INR values in an outpatient anticoagulation clinic setting and to define the therapeutic range for CFX in this population.

Methods

In the present study, INR and corresponding CFX levels were evaluated in 309 randomly selected excess

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specimens collected from the anticoagulation clinic at our medical center. These samples were then deidentified and referenced by random number coding. The testing laboratory had no other demographic information. Also tested were excess plasmas from 30 normal individuals not receiving anticoagulants that had been consented in previous protocols. All of the specimens were collected in 3.2% citrated, vacutainer tubes with a blood to anticoagulant ratio of 9:1. All of the specimens were processed for platelet-poor plasma and stored at −70°C until ready for testing and then rapidly thawed at 37°C immediately before analysis. The specimens were analyzed and then grouped in the following manner: normal donors, anticoagulation clinic patients; INR less than 2.0; INR 2.0–3.0; and INR more than 3.0.

In the present study, the instrument/reagent used was the STA-R Evolution automated coagulation analyzer (Diagnostica-Stago, Inc., Parsippany, New Jersey, USA) and the CFX assay by Diapharma Inc. (Westchester, Ohio, USA). The Diapharma Inc. chromogenic FX kit was the only assay for CFX that was Food and Drug Administration (FDA) approved for clinical use in the United States at the time this protocol was conducted. The INR was performed by a prothrombin time (PT) method using Neoplastine CI+ with an ISI of 1.28 and a geometric mean of 13.8 s. The CFX method was performed by a previously validated method using Diagnostica-Stago, Inc. STA-Unicalibrator, and System N&P controls for factor X.

Data analysis

The relationship ($n = 339$) between CFX and INR assays was assessed using a least squares method and a Marquardt–Levenberg iterative algorithm for a predictive model (Sigmaplot version 9.01; Systat Software, Inc., San Jose, California, USA). The goodness of fit model is expressed as the coefficient of determination (R^2). Receiver–operator characteristic (ROC) curves were employed to assess the ability of CFX to discriminate therapeutic ranges of INR (SPSS version 11.5; SPSS, Inc., Chicago, Illinois, USA). In this study, INR values of less than 2.0 were considered subtherapeutic, an INR of at least 2.0 and 3.0 or less as therapeutic, and an INR of more than 3.0 as supratherapeutic. ROC curve areas of more than 0.900 are considered highly discriminative and more than 0.800 as good discriminators. CFX ranges consistent with INR therapeutic ranges were defined by plots of sensitivity and specificity versus CFX. A Kolmogorov–Smirnov test was employed to assess the normality of the CFX distributions among the therapeutic subsets. Nonnormally distributed data are presented as median and 25th and 75th percentiles. A one-way analysis of variance on ranks (Sigmastat version 3.11; Systat Software, Inc.) was used to assess differences in CFX among INR therapeutic ranges (subtherapeutic: INR <2.0, therapeutic INR 2.0–3.0, supratherapeutic INR >3.0) and differences between groups were assessed using Dunn's post-hoc test. P values of less than 0.05 are considered statistically significant.

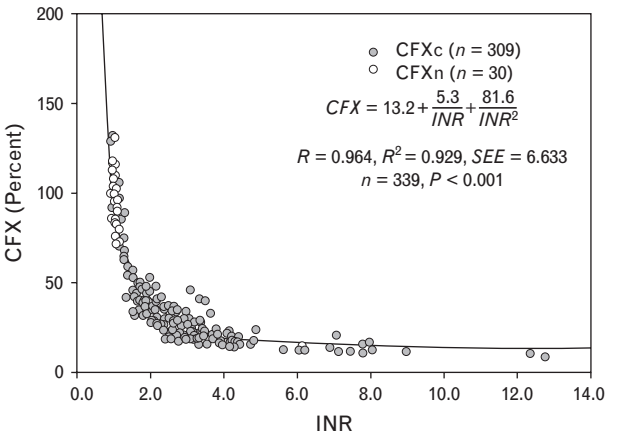
Results

Figure 1 depicts a nonlinear relationship ($R^2 = 0.929$; $P < 0.001$) between INR and CFX assessments derived from the plasma of patients receiving OAT ($n = 309$) and untreated controls ($n = 30$). The ability of CFX levels to discriminate patients with INR of at least 2.0 versus INR of less than 2.0 is shown by the ROC curve (Fig. 2). CFX is highly discriminative for detecting therapeutic from subtherapeutic INR ranges (ROC curve area 0.984, $P < 0.0001$). The assessment of sensitivity and specificity over the range of CFX observed in this study indicate that a CFX of 35.5 or less is equivalent to an INR of at least 2.0 with a sensitivity of 91.7% and specificity of 91.9%.

In the subset of patients with INR values of at least 2.0 ($n = 240$), Fig. 3 illustrates that CFX is a good discriminator between therapeutic and supratherapeutic INR ranges (INR 2.0–3.0 versus INR >3.0). In this subset, the ROC curve area is 0.864 ($P < 0.001$). Plots of sensitivity and specificity in these patients (INR >2.0) indicate that a CFX of 23.5% or less would provide similar results to INR of more than 3.0 for differentiating therapeutic from supratherapeutic ranges of anticoagulation with a sensitivity of 78.2% and specificity of 84.6%.

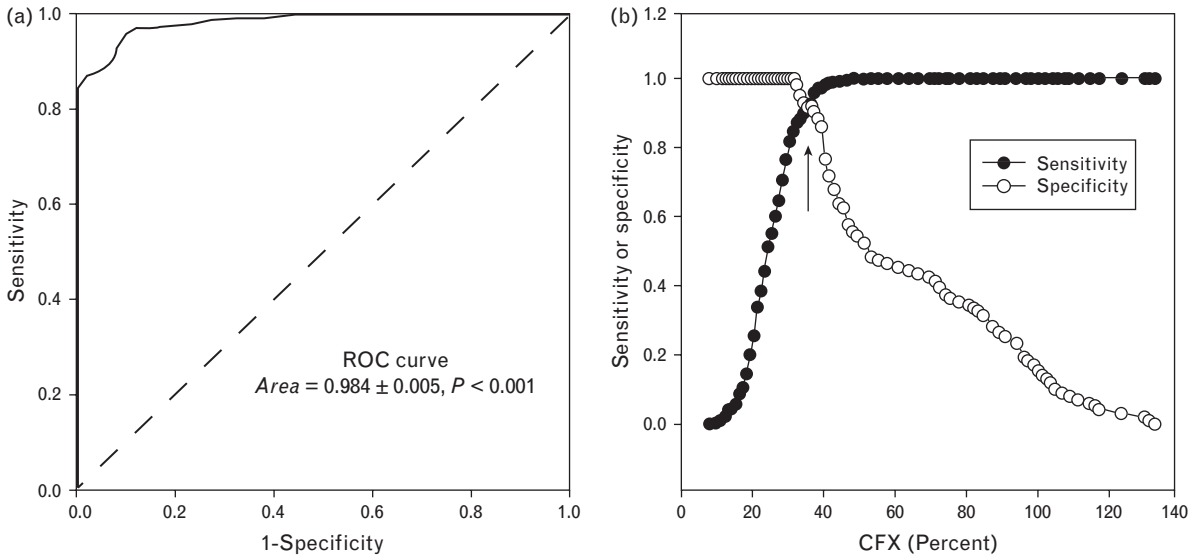
Figure 4 presents CFX data stratified by INR therapeutic ranges: subtherapeutic (INR <2.0), therapeutic (INR 2.0–3.0), and supratherapeutic (INR >3.0). Significant

Fig. 1



A good model fit between INR and CFX when expressed as a second order inverse function ($n = 339$, $R^2 = 0.929$; $P < 0.001$). Open circles represent samples from normal control group (CFXn) and closed circles from patients receiving Coumadin therapy (CFXc). CFX, chromogenic factor X; INR, international normalized ratio.

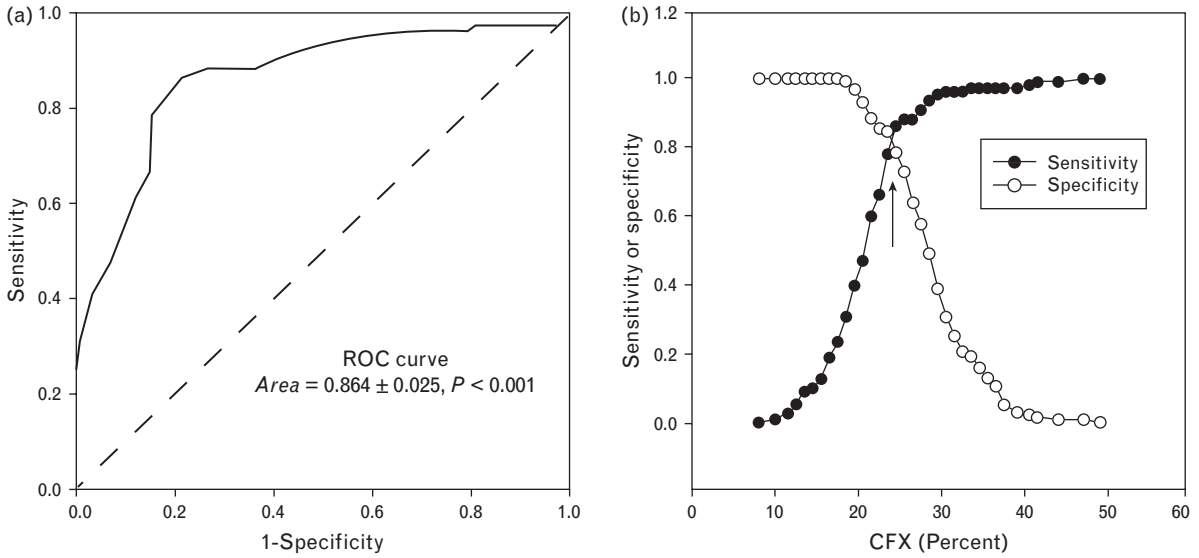
Fig. 2



(a) An ROC curve using INR of at least 2.0 as the criterion for the threshold of therapeutic anticoagulation. (b) (arrow) A plot of sensitivity and specificity over the range of CFX values tested ($n = 339$). The arrow indicates the CFX value of 35.5% or less that has maximum combined sensitivity and specificity for the INR therapeutic threshold (INR $\geq$ 2.0). CFX, chromogenic factor X; INR, international normalized ratio; ROC, receiver–operator curve.

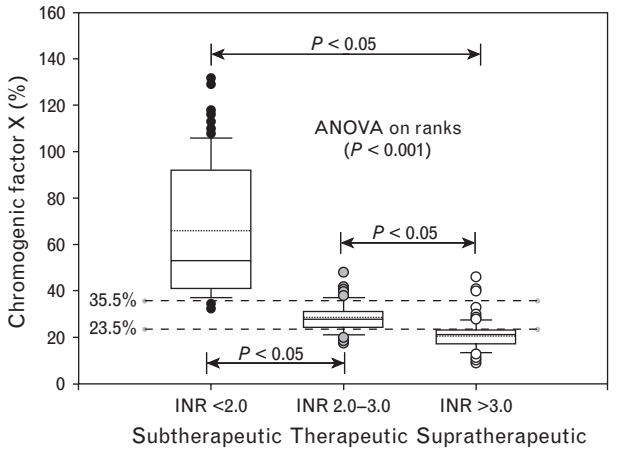
differences in CFX ($P < 0.05$) were noted between all INR therapeutic ranges. The dashed lines indicate CFX values (35.5 and 23.5%) equivalent to the INR therapeutic range (2.0–3.0). Data from two of the sample subsets (INR <2.0 and INR >3.0) were not normally distributed and, therefore, subgroups are compared as box plots indicating the median, 25th and 75th percentiles for each therapeutic range. The CFX values for the therapeutic INR range met the test for normality [mean ± confidence interval (CI) 28.3 ± 10.9%]. The mean and range of the CFX values for each INR therapeutic subgroup are presented in Table 1.

Fig. 3



(a) An ROC curve for the patients with INR of at least 2.0 ($n = 240$) using an INR value of more than 3.0 for discriminating therapeutic from supratherapeutic ranges of CFX. (b) A plot of sensitivity and specificity versus CFX values. Arrow indicates that CFX 23.5% or less is consistent with an INR of more than 3.0. CFX, chromogenic factor X; INR, international normalized ratio; ROC, receiver–operator curve.

Fig. 4



Box plots (median: solid line, mean: dotted line, whiskers: 10th and 90th percentile) for CFX values categorized by INR therapeutic ranges. Significant differences were noted between all groups. Dashed lines indicate the CFX range (23.5–35.5%) is equivalent to the INR therapeutic range (INR 2.0–3.0). ANOVA, analysis of variance; INR, international normalized ratio.

Discussion

The present study is the largest one to date that investigates the use of CFX in monitoring oral anticoagulation. Prior studies have demonstrated the ability of CFX to remain unaffected by the presence of lupus anticoagulant and other variables that affect clottable assays [1–7]. Furthermore, CFX has proven useful in monitoring for therapeutic anticoagulation when converting from argatroban to warfarin. The CFX appears to remain unaffected by argatroban, whereas the INR may be elevated [8–17], making determination of the actual therapeutic level of oral anticoagulation difficult. It appears that CFX may be a useful test for monitoring OAT in broad groups of patients, such as those seen in an anticoagulation clinic.

Prior studies have demonstrated variable ranges of CFX that correspond to therapeutic anticoagulation [8–17], but there is no universally accepted therapeutic range for CFX. Thus, one of our objectives of the current study was to provide further evidence for an optimal range of values of CFX that is considered to be indicative of therapeutic anticoagulation. ROC curves were employed in the present study to discriminate the boundary ranges for CFX consistent with INR therapeutic values (2.0–3.0). This analysis suggests that the CFX range of 23.5–35.5% can be considered therapeutic. The 95% CI for CFX values from samples within the therapeutic INR range was slightly wider and had less discriminative power in our sample population with the instrument/reagent combination used in this study.

Table 1 Chromogenic factor X values defined by international normalized ratio categories

INR	*n*	CFX mean (%)	Min (%)	Max (%)
<2.0	99	65.9	32	132
2.0–3.0	136	28.3	18	48
>3.0	104	20.8	9	46

CFX, chromogenic factor X; INR, international normalized ratio; Max, maximum value in range; Min, minimum value in range; *n*, sample number; OAT, oral anticoagulation therapy. INR less than 2.0, subtherapeutic OAT patients ($n = 69$) and sample individuals not receiving OAT ($n = 30$).

Since 1980, the INR has been considered the gold standard for monitoring oral anticoagulation. Recognizing the limitations of INR, a second objective was to compare the performance of CFX to INR for anticoagulated patients at all ends of the therapeutic spectrum; therapeutic, subtherapeutic, and supratherapeutic INR values. The current study demonstrates a good correlation of the two methods in a randomly selected anticoagulation clinic patient population in which the incidences of factors that may affect the INR are unknown. Therefore, a potential limitation of the present study is using INR as the gold standard for determining therapeutic ranges. If patients such as those with lupus anticoagulant happen to be overrepresented in our patient population, the INR values may be overestimated. However, it is unlikely that the number of these patients present is enough to significantly affect the results for determining the therapeutic range of CFX or the correlation of CFX to INR in the current study.

We did not assess clinical outcomes. Ultimately, further investigation is warranted in larger cohorts of patients on oral anticoagulation to assess the feasibility of using CFX as the primary method of monitoring. Ideally, a study designed to assess clinical outcomes such as thromboembolic events and safety outcomes such as bleeding should be undertaken to compare the INR values with the CFX. Future study could include the comparison with INR in a population that was prospectively screened to eliminate confounding factors for INR. Other issues with CFX to be addressed in further studies include cost, availability of assay, as well as confirmation of the therapeutic range of CFX in a patient population whose INR is screened for confounding factors.

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