Factor X and factor II activity levels do not always agree in warfarin-treated lupus anticoagulant patients
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Objective Warfarin therapy is used in lupus anticoagulant patients with thrombosis and yet the prothrombin time (PT)/international normalized ratio (INR) in these patients can sometimes be falsely elevated. Both a PT-based factor II (FII) assay and a chromogenic, enzymatic factor X (CFX) assay have been used for monitoring when the INR may be artifactual. This study compared FII and CFX assays in lupus anticoagulant-positive and lupus anticoagulant-negative warfarin-treated patients.

Methods Cross-sectional study of samples from 21 lupus anticoagulant-positive and 19 lupus anticoagulant-negative outpatients. Plasma samples were simultaneously measured for FII and CFX and the ratio of FII/CFX was used to measure concordance.

Main results Compared with lupus anticoagulant-negative patients 14 of the 21 lupus anticoagulant-positive patients had lower FII/CFX ratios ($P<0.01$). Three of the patients had ratios less than 0.6 indicating strong disagreement ($P<0.0001$). The patient with the lowest FII/CFX ratio had evidence suggesting a specific antibody to FII. Another patient showed that the discordance between FII and CFX varied over time. The CFX assay in the laboratory was technically superior, more precise, and less costly.

Conclusion The CFX assay is preferred for warfarin therapy monitoring in lupus anticoagulant patients when INR artifacts are suspected. Blood Coagul Fibrinolysis 21:000–000 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Introduction Lupus anticoagulant is an antiphospholipid antibody that may be present in a variety of diseases and may predispose patients to thrombosis that requires warfarin treatment [1]. Warfarin therapy is usually monitored using the international normalized ratio (INR), which is derived from the prothrombin time (PT) clotting test. In some patients and laboratories, the lupus anticoagulant may falsely elevate the INR [2]. Some laboratories and clinicians use a PT-based factor II (FII) assay to monitor warfarin therapy when an INR artifact is suspected [3]. However, because a lupus anticoagulant can affect the PT it may also affect the FII assay. In addition, specific FII antibodies can occur in lupus anticoagulant patients that could cause a falsely low measurement of FII [4–6]. In some institutions, an enzymatic, chromogenic factor X (CFX) assay that avoids clotting test artifacts is used for alternative warfarin monitoring [2,7].

Both the FII and CFX assay results are reported as a percentage of normal factor activity, with pooled normal plasma having 100% activity. The therapeutic range for these assays, corresponding to a therapeutic INR in lupus anticoagulant-negative patients is approximately 20–40% [2,8,9]. We compared the results of CFX and FII in warfarin-treated lupus anticoagulant-positive and warfarin-treated lupus anticoagulant-negative patients to see if the results were interchangeable for monitoring.

Methods This study was reviewed by the Institutional Review Board and met the criteria for exempt review.

Patients All patient care was at the discretion of each patient’s physician. In one hematology clinic it was the practice to monitor all lupus anticoagulant patients taking warfarin with both the CFX and FII assays. We identified 21 patients from this practice who were monitored serially by our reference laboratory. Patients were monitored for a median of 10 months (range 2–36 months) and had a median of 18 paired CFX and FII measurements (range 4–57). No other information about these patients was available to us. All patients had been diagnosed with lupus anticoagulant but not all lupus anticoagulant assays were performed in our laboratory.

For comparison, we selected routine therapeutic INR blood samples from 19 chronic, stable warfarin patients from two of our nonhematology clinics and confirmed that each of these samples was lupus anticoagulant negative; CFX and FII were measured on each of these samples.
Laboratory assays
Blood samples were collected directly into 0.105 mol/l (3.2%) sodium citrate coagulation tubes (BD Vacutainer; Becton Dickinson Company; Franklin Lakes, New Jersey, USA), which were centrifuged at 120s at 8500rpm using the STAT Spin centrifuge (STAT Spin; Inc.; Norwood, Massachusetts, USA). Plasma was sampled in the coagulation analyzer directly from the centrifuged tubes to minimize platelet activation. Although CFX testing was available at all times, some samples were processed into platelet-poor plasma and frozen for the FII assay, as it was only performed during the day.

Confirmatory lupus anticoagulant testing was performed using the Staclot lupus anticoagulant assay (Diagnostica Stago, Asnieres-sur-Seine, France) on a Stago Start four and the lupus anticoagulant SURE dRVVT assay (Precision Biologic, Dartmouth, Nova Scotia, Canada) on a Stago Compact instrument. If either one of these confirmatory tests was positive, a patient was considered to be lupus anticoagulant-positive.

CFX was measured on the STA-R Evolution (Diagnostica Stago; Parsippany, New Jersey, USA) using the DiaPharma Factor X Kit that uses the amidolytic substrate S-2765 and Russell’s viper venom as an activator (DiaPharma; West Chester; Ohio, USA). A standard curve was created using serial dilutions of Unicalibrator reference plasma (Diagnostica Stago; Parsippany, New Jersey, USA) containing a known amount of factor X. The resulting optical density is directly proportional to the amount of factor X. The CFX assay in our laboratory had a coefficient of variation of 2–3%. The lowest reportable CFX result was 5%.

FII assays were performed on the STA-R Evolution using a modified PT assay with Neoplastin CI plus reagent (Diagnostica Stago; Parsippany, New Jersey, USA). A standard curve was created using serial dilutions of Unicalibrator reference plasma (Diagnostica Stago; Parsippany, New Jersey, USA) containing a known amount of FII. The patient sample was mixed with commercially available FII-deficient plasma (Precision Biologic; Dartmouth, Nova Scotia, Canada) and the resulting PT was compared against the standard curve. The degree of correction of the PT was proportional to the activity of FII in the patient’s plasma. The FII assay in our laboratory had a coefficient of variation of 3–6%. The lowest reportable FII result was 7%.

Results
We used the ratio of FII to CFX as the primary measure of assay concordance with a ratio below 1.0 indicating a sample in which the FII was lower than the CFX. Figure 1 shows the distribution of FII/CFX ratios for all patients. The lupus anticoagulant-negative patients had a median ratio of 0.96, a range of 0.58–1.68, and the middle 80% of values between 0.73–1.22. Fourteen of the 21 lupus anticoagulant-positive patients had lower FII/CFX ratios (P for difference < 0.001). Six patients had median ratios less than 0.75 (P for difference < 0.001) with three of the ratios under 0.6.

Figure 2 shows the FII/CFX ratios for patient 03 who had 39 paired measurements over 24 months with a median FII/CFX ratio of 0.32. The sudden peak in the ratio occurred when the patient was not on warfarin for a short time. On the last sample in this patient’s series the lupus anticoagulant was confirmed as positive and the patient had a CFX of 29%, a FII of 13%, and a FVII of 30%. As both the FII and FVII assays are based on the PT, the discrepancy between these two factors in this patient infers that the low FII result was not due to general
interference of the lupus anticoagulant with the PT assay but to something specific for FII.

Figure 3 shows the FII/CFX ratios for patient 04 who had 47 paired measurements over 11 months. There were two distinct periods when the FII assay became discrepant. We do not know anything about the treatment of this patient over the time period. On the last sample in this patient’s series the lupus anticoagulant was negative and all three assays were concordant with a CFX of 29%, a FII of 35%, and a FVII of 35%.

Discussion
Activity of factors II and X are reduced by warfarin treatment but because they have different half-lives in the body, they may not always be at the same level at any point of time. The results in warfarin-treated, lupus anticoagulant-negative patients in this study showed that as the ratio of FII/CFX centered on 1.0, there was a variability that ranged from 0.75 to 1.25 in about 80% of the patients. In contrast, the majority of warfarin-treated, lupus anticoagulant-positive patients in this study had lower FII/CFX ratios with six of the 21 patients having discrepancies in FII that would clearly impact their warfarin dose.

There are two main possibilities for this discrepancy. The first is that the lupus anticoagulant itself interfered with the PT assay used to measure FII. The second possibility is that some lupus anticoagulant patients had factor II antibodies (4–6). In patient 03 in this study, who had the greatest discrepancy between FII and CFX, the finding that the FVII result, which is based on the same PT assay used to measure FII, agreed with the CFX suggests that the patient had a FII antibody. In contrast, patient 04 demonstrated concordance of all three assays on a final plasma sample. However, the concordance between FII and CFX in this patient varied remarkably over time. Because we did not measure FVII in all lupus anticoagulant-positive patients we cannot say that all discrepant FII and CFX results were due to FII antibodies. The lupus anticoagulant itself may have lowered the FII results in some patients. We also did not have serial lupus anticoagulant results on patient samples, so we could not correlate the strength of lupus anticoagulant positivity with the FII/CFX discrepancy.

There are several technical advantages in our laboratory favoring the CFX assay over the FII assay. Not only is the precision of the CFX assay superior to that of the FII assay but also the stability of the CFX reagents greatly exceeds that of the FII reagents. This allows us to quickly offer the CFX assay at all times, something we cannot do with the FII assay. Also, the overall cost of performing the CFX assay is less than that of the FII assay.

In conclusion, the PT-based FII assay produces importantly lower results than the enzymatic CFX assay in many lupus anticoagulant patients. This may be due either to additional antibodies to FII or to a general effect of the lupus anticoagulant on the FII assay. In the laboratory, the CFX assay is superior from a technical and cost standpoint. The CFX assay is preferred over the FII assay when evaluating warfarin therapy in lupus anticoagulant patients who may have INR artifacts.

References

