

Blood Coagulation, Fibrinolysis and Cellular Haemostasis

Point-of-care testing of the international normalized ratio in patients with antiphospholipid antibodies

Stephanie L. Perry¹, Gregory P. Samsa², Thomas L. Ortel^{1,3}

¹Division of Hematology, Department of Medicine, ³Department of Pathology, and ²Center for Clinical Health Policy Research, Duke University Health System, Durham, North Carolina, USA

Summary

Antiphospholipid antibodies can influence the results of clotting tests in a subset of patients, which can be a major obstacle in monitoring warfarin. The aim was to determine if point-of-care testing of the International Normalized Ratio (INR) is influenced by antiphospholipid antibodies. We compared 59 patients receiving warfarin for a diagnosis of antiphospholipid antibody syndrome (APS) to 49 patients receiving warfarin for atrial fibrillation to evaluate the consistency between INR results obtained by different methods. INR results obtained by finger stick (capillary whole-blood) and venipuncture (non-citrated and citrated whole-blood) were compared with our laboratory plasma-based prothrombin time assay. Five patients (8%) with APS and both elevated anti- β_2 glycoprotein I levels and positive lupus anticoagulants had non-measurable ProTime[®] INR results and generally higher Hemochron[®] Signature INR results than the plasma-

based method, but the corresponding chromogenic factor X results were not supratherapeutic. For the remaining patients, differences between the plasma-based INR and the point-of-care INR results ranged from 0.2 ± 0.2 to 0.4 ± 0.3 . The differences were similar for patients with APS and atrial fibrillation for all INR comparisons with the exception of the plasma-based method compared with the ProTime, which showed a mean absolute difference of 0.4 ± 0.3 for APS patients and of 0.2 ± 0.2 for atrial fibrillation patients ($p=0.02$). In a subset of APS patients, the ProTime[®] system will not yield an INR result and the HEMochron Signature (citrated and non-citrated whole-blood) INR results will exhibit elevated INR results. For this subset of APS patients, we suggest using an alternative method to monitor warfarin.

Keywords

Antiphospholipid antibodies, antiphospholipid antibody syndrome, international normalized ratio, point-of-care testing, prothrombin time, warfarin

Thromb Haemost 2005; 94: 1196–202

Introduction

The degree to which antiphospholipid antibodies influence INR results is controversial. We (1, 2) and others (3, 4) have demonstrated that a subset of patients with lupus anticoagulants (LA) have antibodies that influence the prothrombin time (PT) which leads to International Normalized Ratio (INR) results that do not accurately reflect the true anticoagulant effect of warfarin. Sanfelippo, et al. (3) estimated this to occur in 6.5% of patients based on failure of LA-positive patients to correct the PT with normal plasma compared to LA-negative patients. We (2) found that 19% of patients (12/65) with antiphospholipid antibody syndrome (APS) on warfarin had INR results that overestimated the

anticoagulant effect and that of these patients, 11% had INR results that were considered “therapeutic” ($INR > 2.0$), but were subtherapeutic by a chromogenic factor X assay. Also, in a study comparing two different PT assays, Della Valle et al. (4) found that in 41% (8/17) of LA-positive patients on warfarin, INR values were significantly higher using a recombinant versus a combined thromboplastin reagent.

In contrast, others (5–8) have concluded that INR results can be followed in LA-positive patients, as long as LA-sensitive thromboplastins are not used in PT assays (6–8). Of the thromboplastin reagents that have been tested for sensitivity to LA, Innovin and Thromborel R have been shown to be influenced by the presence of LA. Using eight different thromboplastin reagents

Correspondence to:
Stephanie L. Perry, MD
Hemostasis & Thrombosis Center
Duke University Health System
Box 3422, Room 0563 Stead Building
Durham, North Carolina
27710 USA
Tel.: +1 919 684 5350, Fax: +1 919 681 6160
E-mail: perry052@mc.duke.edu

Received June 6, 2005
Accepted after resubmission September 14, 2005

Financial support:
This research was conducted in the General Clinical Research Clinic at Duke University Health System (grant# MO1-RR-30, National Center for Research Resources, General Clinical Research Centers Program, National Institutes of Health) and was supported by the International Technidyne Corporation, ITC, Edison, NJ. Dr. Perry received support from a K-12 award (grant# RR17630-03).

with the International Sensitivity Index (ISI) ranging from 1.0 to 1.8, Robert et al. (7) reported that only Innovin (ISI = 1.10) overestimated the INR in 14% of LA samples (6/43). Similarly, Tripodì et al. (8) found that PT assays using Thromborel R (ISI = 1.33) were significantly influenced by LA with 67% of LA samples (39/58) yielding higher INR results.

To overcome the difficulties associated with LA-sensitive thromboplastins, alternative approaches to monitoring warfarin for patients with APS have been proposed (1, 3, 4). We (1), and Sanfelippo et al. (3) demonstrated that results from the chromogenic factor X assay could provide an alternate method of monitoring the anticoagulant effect of warfarin in patients who have LA that interfere with the PT assay. However, the chromogenic factor X assay is not readily available for most clinical settings due to the need for a specialized coagulation laboratory. Della Valle et al. (4) concluded that accurate INR results may be obtained from combined thromboplastin reagents that permit testing at high plasma dilution, although these reagents are not widely used for INR monitoring.

Point-of-care testing systems, using either recombinant or conventional thromboplastin, are now available through the use of dry-reagent technology. An increasing number of anticoagulation clinics are using point-of-care instruments for monitoring patients on warfarin. It is unknown how this different methodology for PT testing will be affected by samples from patients with APS. We conducted this study to compare the INR results obtained in patients with APS on warfarin by performing our institution's plasma-based method, different point-of-care testing systems, and the chromogenic factor X in order to determine if antiphospholipid antibodies influence INR results obtained by point-of-care testing systems.

Materials and methods

This was a single center, observational study approved by the institutional review board and performed in the General Clinical Research Center (GCRC) at Duke University Health System. Informed consent was obtained on all patients and the Principles of the Declaration of Helsinki regarding investigations in humans were observed.

Antiphospholipid antibody syndrome group and control group

Patients were identified through the Duke Anticoagulation Clinic where approximately 600 patients are followed for management of warfarin. Of these patients about 10% have a diagnosis of APS and about 30% have a diagnosis of atrial fibrillation. For this study, the APS group included patients who have had a positive laboratory test for antiphospholipid antibodies and were on warfarin for a past thrombotic event. The control group included patients who are on warfarin for atrial fibrillation.

Patients were contacted prior to a scheduled appointment for routine monitoring of their INR and asked to have a finger stick performed in addition to the usual venipuncture for the INR result. A citrated-blood sample from the venipuncture was sent to the Duke Coagulation Laboratory for routine INR testing using a plasma-based method. Warfarin doses were adjusted by pro-

viders in the Anticoagulation Clinic based on the plasma-based INR result.

Blood collection and coagulation tests

The two point-of-care testing systems used in this study were the ProTime (International Technidyne Corp, [ITC], Edison, USA) and Hemochron *Signature* (ITC).

For the ProTime INR, capillary blood samples were obtained by a finger stick using a disposable Tenderlett® Plus (incision and collection) device. The ProTime cuvette has five channels which contain dried human recombinant thromboplastin with an ISI of approximately 1.0. Three channels are used to test the PT in triplicate with the median result displayed. Each cuvette also has two levels of controls performed simultaneously on each test. In addition to the reagent, the level I control channel contains dried purified plasma-extracted coagulation factors to normalize the patient's INR, and the level II control channel contains a known amount of anticoagulant to yield an abnormal INR. The quality control system is programmed to display an error message if the control range limits for either control or for the relationship between the level I and level II controls are not met. INR results were performed in duplicate using two different ProTime instruments from one or two finger sticks.

For the Hemochron *Signature* INR, disposable reagent cuvettes for citrated whole blood and non-citrated whole blood were used. The Hemochron *Signature* cuvette uses a thromboplastin of rabbit brain origin with an ISI of 1.0. Since these cuvettes do not have internal controls, both a normal and abnormal control made of non-human animal blood, were performed for each new box of cuvettes. Daily quality control testing was performed on the Hemochron *Signature* instruments using a temperature verification cartridge and two levels of electronic controls. For each INR performed using the Hemochron *Signature* instrument, 50 µl of blood was pipetted into the cuvette sample well and duplicated on a second instrument.

Blood samples collected from a single venipuncture included a discard tube, one blood collection tube without additives, and two tubes with 3.8% trisodium citrate. First, blood collected in the tube without additives was used to obtain an INR result from the Hemochron *Signature* instrument using cuvettes for non-citrated whole blood. This was performed in accordance with our GCRC's specific guidelines for blood collection, although the manufacturer recommends using a syringe to collect the non-citrate whole blood sample. Then, blood collected in a citrated tube was used to obtain an INR result from the Hemochron *Signature* instrument using cuvettes for citrated whole blood.

Citrated blood was also sent to the Coagulation Laboratory and parallel INR measurements were obtained by a plasma-based method using an MDA-180® analyzer (bioMérieux, Durham, USA) with Simplastin-HTF (bioMérieux, Durham, USA), a human cell line thromboplastin with an ISI of 1.15. All samples were stored at -70°C until testing for antiphospholipid antibodies and the chromogenic Factor X could be performed.

Assays

Antiphospholipid antibodies including anticardiolipin IgG (cut-off ≥ 11 GPL units/ml) and IgM (cut-off ≥ 9 MPL units/ml) antibodies, anti-prothrombin antibodies (cut-off ≥ 10 GAU/ml), and

anti-β₂glycoprotein I (anti-β₂GPI) antibodies (cut-off > 0.4 GAU/ml) were detected by ELISA using Asserachrom[®] kits (provided by Dr. Laura Worfolk, Diagnostica Stago, Asnières, France).

Lupus anticoagulants were detected by using the dilute Russell's Viper Venom Time Screen and Confirm (cut-off ≥1.4;

Perquik[®] LA-Check, bioMérieux, Durham, USA) and also the hexphase assay (cut-off > 8 seconds; Staclot[®] LA 20, Diagnostica Stago) to increase the sensitivity of detecting LA positive patients (9). This was in accordance with criteria for LA detection established by the International Society of Thrombosis and Haemostasis (10, 11).

Chromogenic Factor X levels were measured on a Coag a Mate MTX[®] analyzer, using a DiaPharma[®] Factor X kit (diaPharma, West Chester, USA). INR values of 2.0 to 3.0 have been found to correlate to chromogenic factor X levels of 45% to 24%. (Ortel TL, unpublished data). In this study, chromogenic factor X levels were used as a reference against which INR results from different methods were assessed.

Table 1: Patients' characteristics.

| | APS (n=52) | AF (n=46) | p value |
|----------------------------------|------------|------------|---------|
| Age in years | | | |
| Mean (±SD) | 54 (±15) | 71 (±10) | <0.0001 |
| Range | 24–83 | 50–84 | |
| No. of Female | 32 (62%) | 20 (43%) | 0.07 |
| No. of African-American | 10 (19%) | 8 (17%) | NS |
| Elevated Anti-β ₂ GPI | 28 (35%) | 0 (0%) | <0.0001 |
| Elevated Anti-prothrombin | 1 (2%) | 0 (0%) | NS |
| Elevated Anticardiolipin IgG | 17 (33%) | 7 (15%) | 0.04 |
| Elevated Anticardiolipin IgM | 2 (4%) | 4 (9%) | NS |
| Lupus Anticoagulant Positive | 26 (50%) | 3 (7%) | <0.0001 |
| ProTime [®] INR | | | |
| Mean (±SD) | 2.8 (±0.9) | 2.5 (±0.7) | 0.07 |
| Range | 1.1–5.9 | 1.1–4.1 | |
| Hr.Sig (non-citrate) INR | | | |
| Mean (±SD) | 2.8 (±0.8) | 2.7 (±0.6) | NS |
| Range | 1.5–6.2 | 1.5–4.7 | |
| Hr. Sig (citrate) INR | | | |
| Mean (±SD) | 2.7 (±0.8) | 2.6 (±0.5) | NS |
| Range | 1.4–4.8 | 1.2–3.7 | |
| Plasma-based INR | | | |
| Mean (±SD) | 2.7 (±0.8) | 2.4 (±0.5) | 0.05 |
| Range | 1.3–5.0 | 1.1–3.3 | |
| C Chromogenic factor X (%) | | | |
| Mean (±SD) | 36% (±11) | 37% (±13) | NS |
| Range | 19%–77% | 23%–93% | |

APS: antiphospholipid antibody syndrome; AF: atrial fibrillation; No.: number; NS: non-significant; INR: International Normalized Ratio; SD: standard deviation; Anti-β₂GPI: anti-β₂glycoprotein I; Ig: immunoglobulin; Hr.Sig: HemoChron[®] Signature.

Table 2: Summary of 5 patients with APS for whom the Pro-Time[®] would not yield an INR result.

| Patient | 1 | 2 | 3 | 4 | 5 |
|------------------------------------|------|-----|-----|-----|-----|
| Anti-β ₂ GPI (GAU/ml) | 74 | 84 | 46 | 38 | 338 |
| Anti-prothrombin IgG (GAU/ml) | 0.9 | 32 | 9 | 2 | 2 |
| Anticardiolipin IgG (GPL units/ml) | 19 | 107 | 37 | 85 | 286 |
| Anticardiolipin IgM (MPL units/ml) | 0.07 | 16 | 23 | 10 | 4 |
| dRVVT Confirm | 1.8 | 1.9 | 1.7 | 1.9 | 2.1 |
| Staclot [®] LA (seconds) | 22 | 81 | –37 | 70 | 4 |
| Hr.Sig (non-citrate) INR | 5.6 | 4.3 | 6.0 | 3.9 | 4.5 |
| Hr.Sig (citrate) INR | 3.9 | 3.3 | 5.2 | 3.4 | 3.3 |
| Plasma-based INR | 2.9 | 3.2 | 4.5 | 3.3 | 2.8 |
| Chromogenic factor X | 32% | 32% | 20% | 28% | 35% |

APS: antiphospholipid antibody syndrome; INR: International Normalized Ratio; Anti-β₂GPI: anti-β₂glycoprotein I; Ig: immunoglobulin; Hr.Sig: HEMOCHRON[®] Signature; dRVVT: dilute Russell's Viper Venom Time; LA: Lupus Anticoagulant. Cutoffs: Anti-β₂GPI (GAU/ml) > 0.4; Anti-prothrombin IgG (GAU/ml) ≥10; Anticardiolipin IgG (GPL units/ml) = 1; Anticardiolipin IgM (MPL units/ml) ≥9; dRVVT Confirm ≥1.4; Staclot[®] LA > 8 seconds (difference between the two clotting times) Therapeutic range: Chromogenic factor X 24% – 45% (Range for INR values 3.0–2.0).

Statistical analysis

Agreement between INR methods was evaluated by a “difference plot”, known as a Bland-Altman plot (12, 13). This graphical method plots the difference between two tests being compared on the y-axis and the mean of the two tests on the x-axis. Agreement was also evaluated by calculating the absolute difference in INR results between two INR methods and using the mean of this absolute difference to make comparisons between the two patient groups. The mean absolute difference was also evaluated by INR range and further comparisons between the patient groups were made by calculating the percentage of the absolute difference falling within 0.4. Methods were considered to differ clinically if the means of absolute differences between individual methods of obtaining INR results differed by 0.4 or greater. Reproducibility for each point-of-care method was evaluated by calculating the mean absolute difference of duplicate INR results. Comparisons between the two patient groups were made by using two-sample t-tests for means for continuous variables and chi-square test for categorical variables.

We also assessed the utility of using the chromogenic factor X by comparing concordant INR results between all methods for the INR ranges of <2.0, 2.0 to 3.0, and >3.0 with the corresponding chromogenic factor X result.

All statistical analyses were performed using the Statistical Package for SAS[®] version 9.1(Cary, USA) and figures were generated using Microsoft[®] Excel version 2003.

Results

Between May 2003 and March 2004, 59 patients with a diagnosis of APS and 49 with atrial fibrillation participated in this study. Five patients, including three patients in the atrial fibrillation group and two patients in the APS group, were excluded because of the inability to obtain blood by finger stick. Additionally, five patients in the APS group had non-measurable INR results by the ProTime system. These five patients were excluded from the overall analysis and are discussed in detail below.

Patient characteristics are summarized in Table 1. As expected there were differences in age (p<0.0001) and gender (p=0.07), reflecting an older population with atrial fibrillation and an increased number of women with APS. In general, the mean INR results were slightly higher in the APS group for all methods, with a trend towards statistical significance for the Pro-

Time ($p=0.07$) and the plasma-based ($p=0.05$) INR results between the two groups.

Antiphospholipid antibodies have been reported to vary over time in patients; therefore, patients were tested at the time of enrollment using several commercially available assays. To verify that our groups differed for antiphospholipid antibodies, both APS and atrial fibrillation groups were tested (Table 1). In the APS group, 32 patients were positive for at least one test (61%) compared to 12 patients in the atrial fibrillation group (26%). Patients in the APS group were more likely to have LA ($p<0.0001$), anti- β_2 GPI ($p<0.0001$), and anticardiolipin IgG ($p<0.04$) than patients in the atrial fibrillation group. Of the patients in the AF group who did have antiphospholipid antibodies, most had either low-titer anticardiolipin IgM ($n=4$; range 10–22 MPL) or IgG antibodies ($n=7$; range 15–26 GPL).

In five patients with APS, the ProTime system would not give an INR result, whereas the plasma-based method reported INR values ranging from 2.8 to 4.5 (Table 2). In these five APS patients, there were no technical difficulties in the procedure, yet the ProTime instrument gave an error message for each of these patients, indicating that the PT for the control level I did not normalize as expected. This error indicates that the internal control channel in the ProTime cuvette has failed to fall into the predetermined range and an erroneous INR result is suppressed. For these five patients, agreement between the plasma-based INR results was less with the Hemochron *Signature* (non-citrate) than with the Hemochron *Signature* (citrate) system. The plasma-based INR results appeared to correlate with the chromogenic Factor X activity.

Excluding the five patients with APS described above, reproducibility for each point-of-care system was similar for the two patient groups. The mean absolute difference of duplicate INR results were as follows: (1) Hemochron *Signature* (citrate) 0.2 ± 0.2 and 0.2 ± 0.1 ; (2) ProTime 0.3 ± 0.2 and 0.2 ± 0.2 ; and (3) Hemochron *Signature* (non-citrate) 0.3 ± 0.3 and 0.3 ± 0.2 for the APS group and the atrial fibrillation group, respectively.

Agreement between the plasma-based method and the point-of-care systems was assessed by comparing the absolute difference between INR values. Table 3 shows the means (\pm SD) for the absolute difference between INR values for each method being compared between the APS and atrial fibrillation patient groups. The two patient groups were significantly different for the mean absolute differences between INR values for the plasma-based and the ProTime methods ($p=0.02$). Mean INR versus difference plots of the plasma-based and ProTime methods show graphically that the majority of the differences between the two methods fall within ± 0.4 for the atrial fibrillation group (Fig. 1; top) compared to a greater number of the differences falling outside of ± 0.4 for the APS group (Fig. 1; bottom). The differences for both groups are distributed evenly around zero with a tendency for greater differences observed with increasing INR.

Agreement between the plasma-based method and the point-of-care systems was also evaluated by INR range, using the plasma-based INR results to establish the ranges (Table 4). The mean absolute difference between the plasma-based INR and each point-of-care method and percentage within 0.4 was determined. For the INR range of 2.0 to 3.0, the mean absolute difference between the plasma-based INR and the ProTime remained 0.4 for

Table 3: Agreement between different INR methods assessed by the absolute differences of the INR results.

| Methods Compared | APS (n=52) | AF (n=46) |
|---------------------------------------|-------------------|-------------------|
| *Plasma-based and ProTime® | | |
| Mean (\pm SD) | 0.4 (± 0.3) | 0.2 (± 0.2) |
| Range | 0–1.3 | 0–1.1 |
| Plasma-based and Hr.Sig (non-citrate) | | |
| Mean (\pm SD) | 0.4 (± 0.3) | 0.4 (± 0.3) |
| Range | 0–1.6 | 0–1.7 |
| Plasma-based and Hr.Sig (citrate) | | |
| Mean (\pm SD) | 0.3 (± 0.2) | 0.3 (± 0.2) |
| Range | 0–1.2 | 0–1.1 |

*p value = 0.02 for the mean absolute difference comparing the APS and AF groups.
 APS: antiphospholipid antibody syndrome; AF, atrial fibrillation;
 INR: International Normalized Ratio; SD, standard deviation;
 Hr.Sig: HEMOCHRON® Signature.

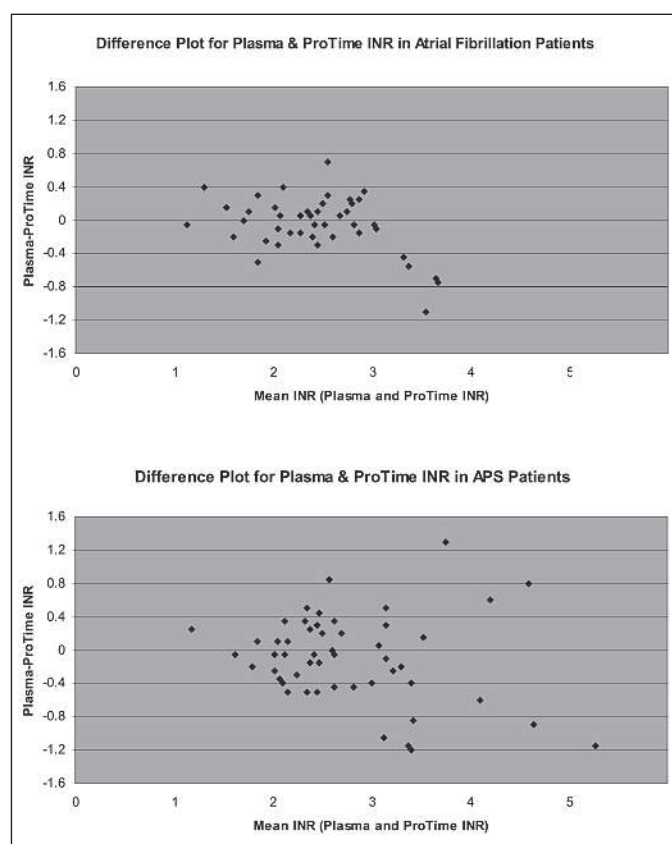


Figure 1: Difference Plot: absolute difference of the plasma-based INR and ProTime. INR on the y-axis; mean INR for the plasma-based INR and ProTime INR on the x-axis. Top: Atrial Fibrillation patients; Bottom: APS patients. INR: International Normalized Ratio APS; antiphospholipid antibody syndrome.

the APS group and 0.2 for the atrial fibrillation group. Better agreement was observed between the plasma-based and ProTime for the atrial fibrillation group with 91% of the mean absolute difference within 0.4, compared to only 66% in the APS group.

Agreement between the INR results in which all methods showed concordance in the ranges of < 2.0 , 2.0 to 3.0 , and > 3.0 with the chromogenic factor X was evaluated (Table 5). In both

Table 4: Agreement between different INR methods as a function of increasing INR assessed by the absolute differences of INR values between the different methods and the percent within 0.4.

| Methods Compared | INR range | APS (n=52) | | | AF (n=46) | | |
|---|-----------|------------|-----------------|------------------------|-----------|-----------------|------------------------|
| | | N | Mean Difference | % within 0.4 INR units | N | Mean Difference | % within 0.4 INR units |
| Plasma-based and ProTime® | <2.0 | 9 | 0.2 | 89% | 9 | 0.2 | 89% |
| | 2.0–3.0 | 29 | 0.4 | 66% | 32 | 0.2 | 91% |
| | 3.1–4.0 | 9 | 0.3 | 80% | 5 | 0.6 | 20% |
| | >4.0 | 5 | 1.0 | 0% | 0 | n/a | n/a |
| Plasma-based and Hemochron (non-citrated) | <2.0 | 9 | 0.2 | 78% | 9 | 0.4 | 67% |
| | 2.0–3.0 | 29 | 0.3 | 72% | 32 | 0.4 | 63% |
| | 3.1–4.0 | 9 | 0.3 | 78% | 5 | 0.5 | 60% |
| | >4.0 | 5 | 0.9 | 20% | 0 | n/a | n/a |
| Plasma-based and Hemochron (citrated) | <2.0 | 9 | 0.2 | 100% | 9 | 0.2 | 100% |
| | 2.0–3.0 | 29 | 0.2 | 83% | 32 | 0.3 | 78% |
| | 3.1–4.0 | 9 | 0.4 | 78% | 5 | 0.4 | 40% |
| | >4.0 | 5 | 0.6 | 20% | 0 | n/a | n/a |

APS: antiphospholipid antibody syndrome; AF: atrial fibrillation; INR: International Normalized Ratio; Hr.Sig: Hemochron® Signature.

Table 5: Agreement between INR results and Chromogenic factor X assessed by INR range.

| | INR | APS | | | AF | | |
|--|---------|-----|--------------|-------------|----|-------------|-------------|
| | | N | CFX mean ±SD | % agreement | N | CFX mean±SD | % agreement |
| Concordant INR results for all methods | <2.0 | 3 | 67% (±8) | 100% | 2 | 74% (±27) | 100% |
| | 2.0–3.0 | 19 | 36% (±6) | 90% | 19 | 33% (±5) | 100% |
| | >3.0 | 11 | 27% (±4) | 18% | *1 | 29% (±n/a) | 0% |

* AF subject with Anticardiolipin IgG 16 units/ml; CFX = Chromogenic Factor X.

the APS and AF group, the chromogenic factor X results were in agreement for the INR results < 2.0. For the INR range of 2.0 to 3.0, the agreement was good for both groups, with only 2 subjects in the APS group having subtherapeutic chromogenic factor X results at 47% and 48%. For the INR range >3.0, the agreement with the chromogenic factor X was poor. Only 2/9 APS subjects had concordant chromogenic factor X results at 19% and 21%; whereas, only one AF subject did not show concordance and interestingly had a low positive anticardiolipin IgG.

Discussion

Warfarin is effective in decreasing recurrent thrombosis in patients with APS; however, the optimal target INR is controversial (14–17). This controversy may be in part be due to the potential influence of antiphospholipid antibodies on the thromboplastin reagents used in PT assays (1–8). In previous studies, we found that INR results do not accurately reflect the true anticoagulant effect of warfarin in a subset of LA-positive patients (1, 2). We hypothesized that the dry-reagent technology used in point-of-care testing of the INR would not be influenced by antiphospholipid antibodies.

Several prospective studies have compared point-of-care testing systems to plasma-based methods (18–22). In these studies, agreement of INR results ranged from 60% to 90% within ±0.5 and 89% within ±0.4 INR units. In two other studies, dif-

ferent point-of-care testing systems were compared to several different thromboplastin reagents using the plasma-based PT assays (23, 24). Both studies found that the different thromboplastins used on the same samples did not yield equivalent INR results with the high-sensitivity thromboplastins (low ISI) giving INR results with less variability then the low-sensitivity thromboplastins (high ISI). The performance of the point-of-care testing systems in these two studies was felt to be satisfactory. None of these studies evaluated patients with APS.

To our knowledge this is the first study to evaluate the use of point-of-care testing systems in patients with a diagnosis of APS. Our study recruited patients on the basis of their diagnosis in the Anticoagulation Clinic. At the time of enrolment, 61% of the patients in the APS group and 26% of patients in the atrial fibrillation group were positive for one or more of the antiphospholipid antibody tests. However, in the atrial fibrillation group, only 3 patients were LA positive (7%) versus 26 in the APS group (50%). In addition, although 11 patients with atrial fibrillation had elevated anticardiolipin antibody levels, none had a titer >30 GPL, and none had elevated anti-β₂-glycoprotein I or antiprothrombin antibody levels. Given the fact that antiphospholipid antibodies are more frequently detected in older patient populations, we felt that analyzing the data by group was appropriate.

In 8% of the APS patients, the ProTime system was unable to determine an INR result (Table 2). In these patients, the control level I channel on the ProTime cuvette did not normalize. This

frequency is consistent with published reports of a subset of APS patients who's PT failed to normalize with the addition of normal plasma (3). In the ProTime system, this failure to normalize leads to suppression of the INR result. There was also wide variation in the INR results obtained by the other methods in this subset. Without ProTime INR results, five of these patients were not included in the overall analysis which may represent a limitation in detecting the influence of antiphospholipid antibodies on point-of-care testing systems.

We also evaluated the agreement between our institution's plasma-based INR to the parallel INR results obtained by two point-of-care testing systems. For the INR range of 2.0 to 3.0, the percentage of INR results within ± 0.4 between the ProTime and the plasma-based methods was only 66% for the APS group and was 91% for the AF group, whereas for the Hemochron *Signature* (citrate) both groups showed similar agreement with about 80% of the INR results within ± 0.4 of the plasma-based method (Table 4).

Comparisons between the two groups were also made by evaluating the agreement of the chromogenic factor X results with concordant INR results (Table 5). The chromogenic factor X had similar agreement for both groups for the INR results ranging < 2.0 and between 2.0 to 3.0. The most striking finding was that 9/11 of subjects in the APS group had chromogenic factor X results that were in the therapeutic range even though the corresponding INR results were > 3.0 .

The advantages of point-of-care testing for the INR (25) must be weighed against the decreased agreement between different methods. In our study, the ISI for the reagent used in the plasma-based method (Simplastin-HTF[®], ISI of 1.15) and for the ProTime system (dried human recombinant thromboplastin, ISI of approximately 1.0) were similar. As with plasma-based methods, there have been reports of incorrect calibrations of the ISI for point-of-care instruments (26). However, the reason for the greater difference observed for the ProTime system in the APS group may be similar to the proposed reason for differences ob-

served with Innovin and Thromborel R in previous studies (7, 8). Tripodi et al. (8) concluded that it may be the phospholipid composition that is responsible for the degree of influence that LA has on a particular reagent.

We found good agreement between our institution's plasma-based method and the ProTime system for atrial fibrillation patients, but not as good for APS patients. We were unable to identify whether the antiphospholipid antibody type or titer accounted for the differences observed between the plasma-based and the ProTime methods. However, we did find that in 8% of the APS patients with both elevated anti- β_2 GPI antibodies and positive for LA, the control level I was unable to normalize which may be due to interference from antiphospholipid antibodies (Table 2). This subset of patients also had wide variations in INR results obtained by the Hemochron *Signature* (citrate and non-citrate) system when compared with the plasma-based method.

The results from this study suggest that the ProTime system will not report INR results for some APS patients. This confirms the finding from published reports of a subset of patients having antiphospholipid antibodies which interfere with INR results. Before implementing point-of-care testing systems to test the INR in APS patients, we recommend evaluating for agreement against a thromboplastin reagent with less sensitivity to antiphospholipid antibodies. For a subset of APS patients an alternative method of monitoring the anticoagulation effect of warfarin such as the chromogenic factor X (1) or using combined thromboplastin reagents (4) may be necessary.

Acknowledgements

The authors thank the following: General Clinical Research Clinic at Duke University Health Systems for use of their facilities and staff support; Laura Norfolk, Ph.D at Stago for providing kits to run assays; ITC for use of the point-of-care instruments and funding for technical and statistical support; Mary Pound and Keith Klemp for performing assays; and Soumaya Elrouby, Ph.D, Richard Becker, MD, Gowthami Arepally, MD, and Bunderika Suwanawiboon, MD for providing insightful comments.

References

- Moll S, Ortel TL. Monitoring warfarin therapy in patients with lupus anticoagulants. *Ann Intern Med* 1997; 127: 177–85.
- Ortel TL, Manares C, Moore KD et al. Antiphospholipid Antibodies and Use of the International Normalized Ratio. *Blood* 1998; 92: 42a.
- Sanfelippo MJ, Sennet J, McMahan EJ. Falsely elevated INRs in warfarin-treated patients with the lupus anticoagulant. *WJM* 2000; 62–4, 43.
- Della Valle P, Crippa L, Safa O et al. Interference of lupus anticoagulants in prothrombin time assays: implications for selection of adequate methods to optimize the management of thrombosis in antiphospholipid-antibody syndrome. *Haematologica* 1999; 84: 1064–74.
- Greaves M, Cohen H, Machin SJ et al. Guidelines on the Investigation and Management of the Antiphospholipid Syndrome. *Br J Haematol* 2000; 109: 704–15.
- Lawrie AS, Purdy G, Mackie IJ et al. Monitoring of oral anticoagulant therapy in lupus anticoagulant positive patients with the anti-phospholipid syndrome. *Br J Haematol* 1997; 98: 887–92.
- Robert A, Le Querrec A, Delahousse B et al. Control of oral anticoagulation in patients with the antiphospholipid syndrome – influence of the lupus anticoagulant on international normalized ratio. *Thromb Haemost* 1998; 80: 99–103.
- Tripodi A, Chantarangkul V, Clerici M et al. Laboratory control of oral anticoagulant treatment by the INR system in patients with the antiphospholipid syndrome and lupus anticoagulant: Results of a collaborative study involving nine commercial thromboplastins. *Br J Haematol* 2001; 115:672–8.
- Tripodi A, Chantarangkul V, Clerici M et al. Laboratory diagnosis of lupus anticoagulants for patients on oral anticoagulant treatment: performance of dilute russell viper venom test and silica clotting time in comparison with staclot LA. *Thromb Haemost* 2002; 88: 583–6.
- Brandt JT, Triplett DA, Alving B et al. Criteria for the Subcommittee on Lupus Anticoagulant / Antiphospholipid Antibody of the Scientific and Standardisation Committee of the ISTH. *Thromb Haemost* 1995; 74: 1185–90.
- Wilson WA, Gharavi AE, Koike T et al. International Consensus Statement on Preliminary Classification Criteria for Definite Antiphospholipid Syndrome: Report of an International Workshop. *Arthritis Rheum* 1999; 42: 1309–11.
- Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986: 307–10.
- Westgard JO. Points of care in using statistics in method comparison studies. *Clin Chem* 1998; 44: 2240–2.
- Rosove MH, Brewer PMC. Antiphospholipid thrombosis: Clinical course after the first thrombotic event in 70 patients. *Ann Intern Med* 1992; 117: 303–8.
- Khamashta MA, Cuadrado MJ, Mujic F et al. The management of thrombosis in the antiphospholipid-antibody syndrome. *N Engl J Med* 1995; 332: 993–7.
- Ginsberg JS, Wells PS, Brill-Edwards P et al. Antiphospholipid antibodies and venous thromboembolism. *Blood* 1995; 86: 3685–91.
- Crowther MA, Ginsberg JS, Julian J et al. A Comparison of two intensities of warfarin for the prevention of recurrent thrombosis in patients with the antiphospholipid antibody syndrome. *N Engl J Med* 2003; 349: 1133–8.

18. Le DT, Weibert RT, Sevilla BT et al. The International Normalized Ratio (INR) for monitoring warfarin therapy: Reliability and relation to other monitoring methods. *Ann Intern Med* 1994; 120: 552–8.
19. Kaatz SS, White RH, Hill J et al. accuracy of laboratory and portable monitor international normalized ratio determinations: Comparison with a criterion standard. *Arch Intern Med* 1995; 155: 1861–7.
20. Reed C, Rickman H. Accuracy of International Normalized Ratio determined by portable whole-blood coagulation monitor versus a central laboratory. *Am J Health Syst Pharm* 1999; 56:1619–23.
21. Andrew M, Ansell JL, Becker DM et al. Point-of-Care Prothrombin Time Measurement for Professional and Patient Self-Testing Use: A Multicenter Clinical Experience. *Am J Clin Pathol* 2001; 115: 288–96.
22. Douketis JD, Lane A, Milne J et al. Accuracy of a portable International Normalization Ratio Monitor in outpatients receiving long-term oral anticoagulant therapy: Comparison with a laboratory reference standard using clinically relevant criteria for agreement. *Thromb Res* 1998; 92: 11–7.
23. Tripodi A, Chantarangkul, Bressi C et al. International Sensitivity Index Calibration of the near-patient testing prothrombin time monitor, ProTime. *Am J Clin Pathol* 2003; 119: 241–5.
24. Pierce MT, Crain L, Smith J et al. Point-of-Care versus laboratory measurement of the International Normalized Ratio. *Am J Health Syst Pharm* 2000; 57: 2271–4.
25. Woods K, Douketis JD, Schnurr T et al. Patient preferences for capillary vs. venous INR determination in an anticoagulation clinic: a randomized controlled trial. *Thromb Res* 2004; 161–5.
26. Tripodi A, Arbini AA, Chantarangkul V et al. Are capillary whole blood coagulation monitors suitable for the control of oral anticoagulant treatment by the international normalized ratio? *Thromb Haemost* 1997; 78: 855–8.
-