Each TAS PT-One test cartridge contains lyophilized thromboplastin from human placenta, calcium chloride, and buffers coated with paramagnetic iron oxide particles (PIOP). The citrated blood is added to the single-use test cartridge positioned within the TAS analyzer. A pulsating magnetic field is then initiated, causing the PIOP to move up and down. As the sample clots, the decrease in PIOP motion is detected and the resulting clotting time displayed. Each cartridge lot is magnetically encoded with an International Sensitivity Index (ISI) and a reference mean value, 0.99 and 12.2 s, respectively.

For laboratory determination of PT, we used Innovin (Dade International) thromboplastin, which contains recombinant human tissue factor, calcium chloride, and buffers. All testing was performed on the MLA 1000C coagulation analyzer (Medical Laboratory Automation). The ISI for this method is 0.98, with mean PT of 11.1 s.

Linear regression analysis and bias analysis were performed in terms of the clinical laboratory method serving as the reference. Student’s paired t-test was also used to determine whether the differences between POC devices and the parent clinical laboratory method were statistically significant (P < 0.05).

The summary of data (Table 1 and Fig. 1) indicates only marginal agreement of the clinical laboratory method with the Coumatrak and CoaguChek Plus devices. This is not unexpected, given the sensitivity differences indicated by the ISI. The agreement improved slightly when the methods were compared in terms of INR values, but the differences remained statistically significant.

For both PT and INR, the TAS method agreed more closely with the clinical laboratory method than did the other POC devices (Table 1), especially for samples with INR < 2.5 (Fig. 1). In theory, using INR [1] should have minimized the differences between methods and devices, but we did not find this to be the case here. Indeed, 25% (10 of 40) of the Coumatrak results and 17.5% (7 of 40) of the CoaguChek Plus results indicated adequate anticoagulation (INR 2.0–3.0) [2] when subtherapeutic anticoagulation was reported by the parent clinical laboratory or TAS methods.

From our data we conclude the following: The Coumatrak and CoaguChek Plus methods significantly underestimated the INR. The TAS PT-One is more comparable with the parent laboratory method, both of which use a highly sensitive thromboplastin reagent. Differences between methods were not fully corrected with the INR.

References

Factor X Values as a Means to Assess the Extent of Oral Anticoagulation in Patients Receiving Antithrombin Drugs, Debra A. Hoppensteadt,* Steven Kahn, and Jawed Fareed (Dept. of Pathol., Loyola Univ. Med. Center, 2160 S. First Ave., Maywood, IL 60153; * address correspondence to this author, at Rm. 2643, fax 708 216-6660)

Several antithrombin drugs currently under clinical development for cardiovascular indications include argatro-
ban, efegatran, hirudin, and polyethylene glycol (PEG)-hirudin. As with heparin, these patients are started on warfarin while still being treated with the antithrombin agents until an INR value of 2.5–3.5 is achieved. One problem seen in these patients is that the antithrombin agents interfere in the prothrombin time (PT) assay by inhibiting thrombin and preventing the formation of a fibrin clot, thus causing a falsely increased INR. Therefore, an alternative method is needed to reliably quantify the effect of oral anticoagulant drugs in patients simultaneously administered oral anticoagulants and antithrombin agents.

Oral anticoagulants are known to influence the functional concentrations of the coagulation factors II, VII, IX, and X. Thus, a direct measure of any of these factors may be useful in predicting the extent of anticoagulation with oral anticoagulants [1]. Factor VII and factor IX can be measured only in clot-based assays and, because of interference of the antithrombin agents with the fibrin clot formation, any clot-based assay will produce a falsely prolonged clotting time. However, factor X can be measured by both clot-based and amidolytic-based methods. A modified amidolytic method to determine the suppression of functional factor X concentrations in patients treated with combination therapy (oral anticoagulants and antithrombin agents) has been researched [2, 3]. In this method, Tris buffer (0.05 mol/L, pH 7.8) was supplemented with calcium (25 mmol/L) and recombinant hirudin (2.5 g/L) and mixed with citrated plasma, followed by activation of factor X with Russell’s viper venom. The factor Xa generated was measured with an amidolytic substrate specific for factor Xa. To validate this method, we diluted normal human plasma from 5 male and 5 female donors in factor X-deficient plasma to construct a calibration curve in the range of 0–100% factor X. The effect of different amounts of oral anticoagulation in this method was tested with citrated plasma samples from patients treated only with oral anticoagulants in the specified PT ranges (n = 15–20/group). In these samples, obtained from the coagulation laboratory at Loyola University Medical Center in Maywood, IL (Table 1), a proportional suppression of factor X was observed with increases in PT. When argatroban, 5 g/L, was supplemented in vitro to these samples, reassembly by the amidolytic factor X assay showed no statistical difference between the factor X value observed in the control and that in the samples containing argatroban (Table 1).

In a clinical study in which 20 patients undergoing percutaneous transluminal coronary angioplasty received only argatroban as a 350 μg/kg bolus, followed by a 5–10 μg/kg per minute infusion, the factor X values were 80% ± 15%. Thus, argatroban administered by itself has no effect on the factor X concentration.

In a second clinical study, performed at Loyola University Medical Center, 10 patients who were positive for heparin-induced thrombocytopenia received argatroban in a 2 μg/kg per minute infusion for treatment of thrombosis; later, they were administered therapeutic dosages of warfarin during a 3-day cross-over period. The control group in this study consisted of 5 male and 5 female normal healthy volunteers. The other two groups were the same patients who at first received argatroban only (a) and later were administered warfarin and argatroban (b). These latter patients exhibited a prolonged PT and a marked increase in INR values (range 7–21). Their factor X values measured are shown in Fig. 1 (28% ± 9% NHP), results comparable with the factor X value obtained in the group of patients who had a PT >25 s and did not receive argatroban (Table 1).

These results suggest that oral anticoagulants such as coumadin can be monitored in the presence of a thrombin inhibitor by using the amidolytic factor X assay. However, further studies measuring the factor X values in patients receiving thrombin inhibitors with different concentrations of coumadin are necessary to further validate these claims.

Table 1. Effect of oral anticoagulation and oral anticoagulation plus argatroban on factor X results.

<table>
<thead>
<tr>
<th>PT ranges, s (and INR)</th>
<th>Oral anticoagulants</th>
<th>Oral anticoagulants plus argatroban</th>
</tr>
</thead>
<tbody>
<tr>
<td>10–15</td>
<td>89.9 ± 12.6</td>
<td>86.4 ± 13.9</td>
</tr>
<tr>
<td>15–20</td>
<td>59.7 ± 18.7</td>
<td>51.7 ± 20.4</td>
</tr>
<tr>
<td>20–25</td>
<td>38.7 ± 12.4</td>
<td>38.7 ± 12.4</td>
</tr>
<tr>
<td>&gt;25</td>
<td>29.4 ± 10.7</td>
<td>29.4 ± 10.7</td>
</tr>
</tbody>
</table>

*Argatroban supplemented in vitro at 5 μg/mL. NHP, normal human plasma.

Fig. 1. Factor X values in controls and in patients treated with argatroban or argatroban plus warfarin.

References


Xylum CSA®: Automated System for Assessing Hemostasis in Simulated Vascular Flow, Conan K.N. Li,* Thomas J. Hoffmann, Pei-Ying Hsieh, Suneil Malik, and William Watson (Xylum Corp., 670 White Plains Rd., Scarsdale, NY 10583; *corresponding author: fax 914-725-1158, e-mail Conan_Li@Xylum.ccmail.compuserve.com)

Nearly 20 years ago it became clear that analysis of hemostasis function in blood was affected by sample shear rate and anticoagulation [1]. Yet, present technologies still perform hemostasis analysis on anticoagulated samples under static conditions. In 1984, Gorog and Ahmed developed a test system that analyzed hemostasis function of untreated whole blood under physiological flow [2], a technology potentially useful for assessing patients with bleeding disorders [2, 3]. This approach has been automated in a user-friendly benchtop instrument, the Clot Signature Analyzer (CSA®; Xylum Corp.). From a single venipuncture, the CSA provides information on platelet adhesion induced by high shear, platelet aggregation, and coagulation attributable to humoral factors [4]. Measurements are performed on untreated nonanticoagulated whole blood under the physiological conditions of nonrecirculating flow at 37 °C.

The single-use, disposable CSA cassette consists of two perfusion channels, the “punch” and “collagen” channels. In each channel, blood is perfused in tubing under conditions simulating vascular flow. Blood is drawn into two 3-mL syringes, which are attached to luer fittings on the cassette. When the cassette is loaded onto the instrument, low-density oil is delivered into the syringes from a reservoir on the instrument. The imiscible oil rises in the syringes and displaces the blood, which then flows into perfusion lines maintained at 37 °C. The luminal pressure exerted by the flowing blood is measured continuously in both channels by sensors on the instrument. In the punch channel, vascular injury is simulated by piercing the blood perfusion line with a 0.15-mm-diameter needle to form two small holes. Blood flow is largely diverted through these “punch” holes, causing a sudden decrease in luminal pressure (Fig. 1A). The punch holes close as a result of hemostasis, thereby restoring luminal pressure to its original value (punch recovery in Fig. 1A). The time

![Fig. 1.](https://academic.oup.com/clinchem/article-abstract/43/9/1786/5640999)