The Effect of Dabigatran on Select Specialty Coagulation Assays

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Abstract

Dabigatran etexilate is a new oral anticoagulant that functions as a direct thrombin inhibitor. An inhibitor of thrombin has the potential to interfere with essentially all clot-based coagulation assays and select chromogenic assays, whereas the drug would not be expected to interfere in antigen-based assays. The purpose of this study was to evaluate the effect of dabigatran on various specialized coagulation assays using normal plasma specimens with varying concentrations of dabigatran (the active form of dabigatran etexilate). We have demonstrated that samples containing therapeutic levels of dabigatran may lead to underestimation of intrinsic factor activities with abnormal activated partial thromboplastin time (aPTT) mixing study results and a false-positive factor VIII Bethesda titer; overestimation of protein C and protein S activity and activated protein C resistance ratio when determined using aPTT-based methods; and overestimation of results based on chromogenic anti-IIa assays but no effect on antigen assays and select chromogenic assays.

Dabigatran etexilate (Pradaxa, Boehringer-Ingelheim, Ingelheim, Germany) is an oral anticoagulant approved in the United States and Europe for thromboprophylaxis in orthopedic surgery and atrial fibrillation. Because of the drug's predictable pharmacodynamics, pharmacokinetics, and wide therapeutic window, dabigatran does not require therapeutic monitoring. For these reasons, dabigatran use may be favored over either warfarin and/or heparin administration. It is estimated that 3 million individuals in the United States suffer atrial fibrillation[1] and that approximately 250,000 individuals in the United States undergo hip replacement every year and therefore may be candidates for anticoagulation with direct thrombin inhibitors.[2]

Dabigatran etexilate, an inactive precursor, is rapidly converted by esterase-catalyzed hydrolysis to dabigatran, the active form of the drug. Dabigatran is a synthetic, reversible, competitive, rapidly acting, specific inhibitor of both free and bound thrombin. Peak plasma concentrations are achieved about 2 to 3 hours after oral administration with a terminal elimination half-life of 12 to 17 hours.[3] In the Randomized Evaluation of Long-term anticoagulant therapY (RE-LY) trial, the mean peak plasma concentration in patients who received a 150-mg twice-daily regimen was approximately 180 ng/mL and ranged from approximately 80 to 200 ng/mL (excluding those with a creatinine clearance less than 30 mL/min).[4] A significant complication of dabigatran therapy is minor or major bleeding episodes for which there is currently no known specific antidote.

Individuals taking dabigatran may at some point in their therapy require 1 or more coagulation assays. Such assays may be performed to (1) determine the degree of

anticoagulation in a patient who requires an invasive procedure, (2) assess compliance, (3) assess anticoagulant effect in patients with decreased renal function, (4) determine the level of anticoagulation in patients with a minor or major bleeding episode and uncover an underlying coagulopathy, and (5) evaluate for underlying thrombophilia to help determine whether anticoagulant therapy can be safely discontinued. As would be expected with a thrombin inhibitor, dabigatran has in fact been shown to prolong routine coagulation assays such as prothrombin time (PT), activated partial thromboplastin time (aPTT), and thrombin time (TT) tests.[3,5,6] Less is known about the effect of this drug on special coagulation tests. It is important for both laboratorians and clinicians to understand the effect of dabigatran on coagulation tests so that results can be interpreted appropriately, avoiding mistaken diagnoses and potential therapeutic misadventures. This study was undertaken to examine the effect of varying concentrations of dabigatran on specialty coagulation assays as performed with various testing platforms and methods using dabigatran-enriched normal pooled human plasma.

Methods and Results

Normal plasma samples from 10 fresh frozen plasma units obtained from a local blood collection center were thawed in a 37ºC water bath for 30 minutes, then pooled into a single sterile vessel. Dabigatran was diluted in 1 mol/L of hydrochloric acid and dimethyl sulfoxide to give a final concentration of 1 mg/mL of drug. Ten sterile containers, each containing 40 mL of normal pooled plasma (NPP), were enriched with dabigatran to obtain drug concentrations of 25, 50, 75, 100, 125, 150, 200, 300, 400, and 500 ng/mL. Each concentration of dabigatran-enriched NPP was transferred into cryovials labeled A through K (with sample A representing NPP, sample B with 25 ng/mL dabigatran, etc), with each aliquot containing 1.5 to 2.0 mL of dabigatran-enriched NPP. Once separated into aliquots, the cryovials were stored at –70ºC. A series of 20 centers in the United States, Canada, and the United Kingdom with expertise in coagulation laboratory testing volunteered to participate in a blinded study to assess the effect of dabigatran on coagulation tests performed in their laboratory. Each site was requested to perform specialty coagulation assays of their choosing. Samples labeled from A through L were sent via express delivery in polystyrene foam (Styrofoam, Dow Chemical, Midland, MI) containers with ample dry ice to ensure maintaining a frozen state. The participating laboratory confirmed receipt and sample integrity to the study coordinator (R.G.). Once testing was complete and results returned to the investigator, the testing site was unblinded to the dabigatran concentration.

Analytes Measured

Of 19 participating laboratories that responded, the following assays were performed: inhibitor screen (mixing studies) for both PT (n = 1 laboratory) and aPTT (n = 2); factors II (n = 1), V (n = 1), and VIII (clot-based [n = 3]; chromogenic assay [n = 1]) and factors IX (n = 2), X (n = 2), and XI (n = 1) activities using clot-based methods; factor V (n = 2) and VIII (n = 3) inhibitor assays using the Bethesda titer method (n = 3); quantitative D-dimer (n = 6); reptilase time (n = 1); von Willebrand factor antigen (n = 1); von Willebrand factor activity (ristocetin-based [n = 1]; heparin anti-Xa activity (n = 4); antithrombin activity (n = 4); plasminogen activity (n = 1); protein C activity (clot-based [n = 1]; chromogenic method [n = 2]); clot-based protein S activity (n = 1); free protein S antigen (n = 1); and activated protein C (APC) resistance (n = 1). Assays were performed according to each testing laboratory's standard operating procedures using standard protocol, reagents, and instruments.

Inhibitor Screen Testing

PT mixing studies were performed at one site (Dade Innovin, BCS Classic Siemens Healthcare Diagnostics, Marburg, Germany) using George King pooled normal plasma (George King Bio-Medical, Overland Park, KS). aPTT mixing studies were performed at 2 laboratories using 2 different reagent instrument systems (site 1 using Dade Actin FS, Siemens Healthcare Diagnostics and George King NPP; site 2 using TriniCLOT automated aPTT on MDAII [Tcoag Ireland, Wicklow, Ireland] and CRYOcheck normal plasma [Precision BioLogic, Dartmouth, Canada]. For both PT and aPTT studies, normal plasma mix was performed using 1 part sample and 1 part normal plasma and incubated at 37°C for 60 minutes. Interpretations of mixing studies were at the discretion of the testing site. One site used Rosner index calculations, in which an index less than 15 is indicative of factor deficiency and an index higher than 15 indicating inhibitor.

For immediate PT mixing studies, the Rosner index was higher than 15 for all dabigatran samples containing more than or equal to 300 ng/mL of drug. Incubated PT Rosner index was higher than 15 for all dabigatran samples of more than 150 ng/mL of drug. Immediate aPTT normal plasma mix showed an inhibitor effect as determined by failure to correct into the normal aPTT range or a positive Rosner index evident at about 50 to 75 ng/mL of dabigatran and was consistently evident at all higher drug concentrations. Surprisingly, 37°C incubation for 60 minutes led to marked additional prolongation of the aPTT compared with the immediate mix such that the clotting time after incubation exceeded the baseline aPTT at all concentrations of dabigatran.

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Factor Assays

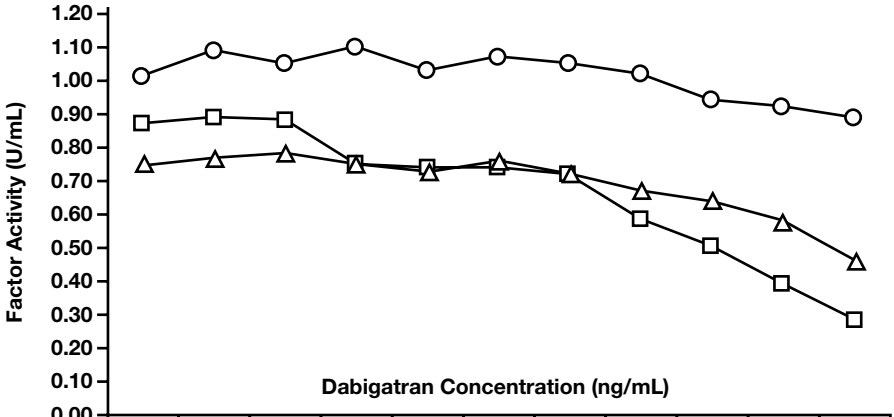
Two sites provided one-stage, PT-based assays for factors II, V, and X activity, at 3 dilutions for each concentration of dabigatran with mean result reported. A drug concentration–dependent and clinically significant decrease in PT-based factor II, V, and X activity was seen, and the effect was significantly greater in the factor II assay **Figure 1**. Factor II activity appreciably changed (>15% decrease in measured activity compared with NPP, sample A results) once dabigatran concentration exceeded 125 ng/mL. For factor V activity, an activity decrease of more than 15% occurred at dabigatran concentrations of 400 ng/mL or more of the drug, whereas the effect of dabigatran on factor X measurements was minimal. The coefficient of variation (CV) among the 3 tested dilutions for each factor assay per sample was less than 15%, regardless of drug concentration, and therefore a nonspecific inhibitor effect was not demonstrated **Table 1**.

Table 1
Effect of Dabigatran on Factor V and Factor VIII 1-Stage Activity Assays[a]

Dabigatran Concentration, ng/mL	Factor V Activity		Factor VIII Activity	
	Mean	CV, %	Mean	CV, %
0	75	4.3	70	5.7
25	77	7.8	62	2.8
50	78	7.3	55	7.3
75	75	5.8	50	8.1
100	73	4.4	43	7.1
125	76	3.3	38	21.1
150	72	3.7	35	24.1
200	67	2.3	28	22.3
300	64	13.0	18	27.8
400	58	11.7	12	38.7
500	46	11.3	7	51.5

CV, coefficient of variation.
[a] Factor activity results are the mean of 3 different dilutions (eg, 1:5, 1:10, and 1:20) for a given sample. Factor activity CV reflects the imprecision of a given sample factor assay result for the 3 dilutions. Acceptable CVs for a given sample in 3 dilutions should be less than 20%. Higher CVs indicate nonparallelism consistent with nonspecific inhibitor effect secondary to dabigatran.

Three laboratories performed 1-stage, aPTT-based factor assays at 3 dilutions for each sample tested. One site chose to analyze factor VIII activity at 9 dilutions for each concentration of dabigatran to determine whether the nonspecific inhibitor effect could be completely diluted. A dabigatran concentration–dependent and clinically significant decrease in aPTT-based intrinsic factor activity was seen beginning at 25 ng/mL of dabigatran **Figure 2**. A more than 15% variation from sample A (sample containing no drug) was seen for samples containing more than 25 ng/mL of drug for 2 sites measuring factor VIII activity and more than 50 ng/mL of drug for a third site (Figure 2). All dabigatran-enriched samples had a more than 15% decrease in factor IX activity from both testing sites, and factor XI activity appreciably decreased with 25 ng/mL or more of the drug. At one testing site, the CV among the 3 tested dilutions for each assay per sample exceeded 20% at 75 ng/mL of drug, whereas at a



	0	25	50	75	100	125	150	200	300	400	500
Factor II	0.87	0.89	0.86	0.75	0.74	0.74	0.72	0.58	0.50	0.39	0.28
Factor V	0.75	0.77	0.78	0.75	0.73	0.76	0.72	0.67	0.64	0.58	0.46
Factor X	1.01	1.09	1.05	1.10	1.03	1.07	1.05	1.02	0.94	0.92	0.89

Figure 1 Effect of dabigatran on prothrombin time (PT)–based factor activity assays. The effect of increasing concentrations of dabigatran mixed with normal plasma on factor II (squares), factor V (triangles), and factor X (circles) activities are shown, where 0 concentration dabigatran represents normal pooled plasma. The PT-based factor assays therefore have the potential to falsely indicate a factor deficiency in a patient with normal levels.

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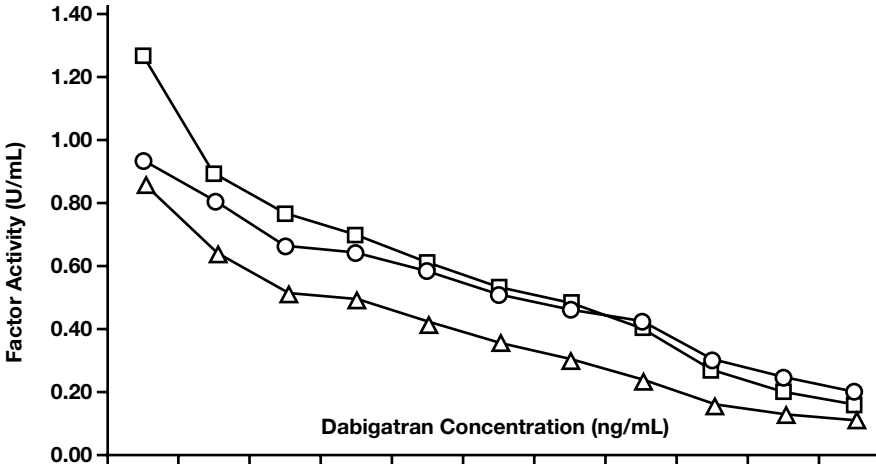

	0	25	50	75	100	125	150	200	300	400	500
Factor VIII	0.87	0.65	0.52	0.50	0.42	0.36	0.31	0.24	0.16	0.13	0.11
Factor IX	1.27	0.89	0.77	0.70	0.61	0.54	0.49	0.40	0.27	0.20	0.16
Factor XI	0.93	0.80	0.66	0.64	0.58	0.51	0.46	0.41	0.30	0.24	0.20

Figure 2 Effect of dabigatran on activated partial thromboplastin time (aPTT)–based factor activity assays. The effect of increasing concentrations of dabigatran mixed with normal plasma on factor VIII (triangles), factor IX (squares), and factor XI (circles) activities are shown, where 0 concentration dabigatran represents normal pooled plasma. The aPTT-based factor assays therefore have the potential to falsely indicate a factor deficiency in a patient with normal levels.

second site, the more than 20% CV threshold was exceeded at 125 ng/mL of dabigatran, thus demonstrating nonparallelism consistent with nonspecific inhibitor effect (Table 1). No additional benefit was seen to diluting samples more than 3 times; despite multiple attempts, dabigatran effect could not be consistently diluted, resulting in factitiously decreased factor activity levels. Factor VIII and IX activities fell below 10% at a concentration of 300 ng/mL or more of dabigatran. Dabigatran concentration had no effect on chromogenic factor VIII activity results.

Table 2 Effect of Dabigatran on Factor VIII Bethesda Assay

Dabigatran Concentration, ng/mL	Recovered Mean Factor VIII Activity, %	Bethesda Units		
		1:1[a]	1:5[a]	1:10[a]
0	>100	0	0	0
25	>100	0	0	0
50	>100	0	0	0
75	>100	0	0	0
100	>100	0	0	0
125	>100	0	0	0
150	90	<0.3	0	0
200	72	0.3	<0.3	<0.3
300	50	1.0	6.3	11.2
400	35	1.6	6.7	11.8
500	18	2.5	5.9	12.6

[a] 1:1, 1:5, and 1:10 represent the dilutions used in the Bethesda assay. Each sample was mixed with normal pooled plasma (NPP), incubated for 1 hour at 37°C, then tested for residual factor VIII activity against a standard curve prepared from NPP/buffered saline. The recovered factor VIII activity result is the mean of 3 different dilutions (eg, 1:5, 1:10, and 1:20) for a given sample. The Bethesda unit 1:1 reflects the reported titer when sample was mixed 1:1 with NPP, and 1:5 and 1:10 are the titers reported when sample was diluted 1:5 and 1:10 with buffered saline before mixing 1:1 with NPP. The lower limit of sensitivity for the test is less than 0.3 Bethesda units, and results with residual factor activity less than 100% but more than 80% are reported in Bethesda units less than 0.3. Increasing dabigatran concentration is associated with increased false positivity for the Bethesda titer method in determining factor VIII inhibitors; the false-positive rate does not improve with predilution of sample with buffered saline.

Bethesda Assay for Inhibitor Titers

Factor V inhibitor assay was performed at one site (Dade Innovin on BCS Classic with immunodepleted factor V–deficient plasma) using George King NPP. Factor VIII Bethesda assay was performed at 2 sites using 2 different instrument/reagent platforms (site 1 using Dade Actin FS, George King NPP, and immunodepleted factor VIII–deficient plasma; site 2 using Dade Actin FS, Precision Biologic CRYOcheck, and George King factor VIII–deficient plasma). No evidence was seen of a dabigatran effect on factor V inhibitor assay because all drug concentrations yielded no evidence of inhibitor. For factor VIII Bethesda titer, evidence of the factitious presence of inhibitor was seen at both sites when the dabigatran concentration was 200 ng/mL or more, with poor reproducibility (CVs >20%) seen among dilutions at higher drug concentrations **Table 2**.

Quantitative D-Dimer

Seven sites used 3 different D-dimer methods: INNOVANCE D-dimer (Siemens Healthcare Diagnostics), STA-Liatest D-Dimer (Diagnostica Stago, Asnieres, France), and HemosIL D-Dimer HS (Instrumentation Laboratory, Bedford, MA). Dabigatran had no effect on D-dimer testing.

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Reptilase

One site evaluated reptilase time (STA-Reptilase) with the STart 4 assay (Diagnostica Stago). This test may be used to differentiate prolonged TTs caused by drug effect (eg, heparin or some thrombin inhibitors) from the presence of a dysfunctional or low fibrinogen. Dabigatran had no effect on reptilase times.

von Willebrand Factor

One site evaluated von Willebrand factor activity using the ristocetin cofactor assay (BC von Willebrand reagent, Siemens Healthcare Diagnostics) and von Willebrand factor antigen using latex immunoassay methods (HemosIL, von Willebrand factor antigen, Instrumentation Laboratories). Dabigatran had no effect on these tests.

Antithrombin Activity

Three different antithrombin assays were performed at 4 different laboratories. Two kits are based on inhibition of thrombin (Berichrom antithrombin III [Siemens Healthcare Diagnostics] and STA-Stachrom AT III 3 [Diagnostica Stago]) and 1 on inhibition of factor Xa (HemosIL liquid antithrombin [Instrumentation Laboratories]). Antithrombin activity assays are chromogenic assays based on the ability of functional antithrombin, in the presence of heparin, to inactivate either thrombin or factor Xa. Thrombin-based antithrombin activity assays overestimated functional antithrombin concentrations in the presence of dabigatran, and this effect is evident at approximately 125 ng/mL, resulting in an approximately 10% increase in concentration compared with baseline **Figure 3**. At 400 to 500 ng/mL dabigatran, the factitious elevation of antithrombin activity was 20% to 30%. The factor Xa–based antithrombin assay was unaffected by the presence of dabigatran.

Plasminogen Activity

One laboratory evaluated plasminogen activity using a chromogenic method, Berichrome plasminogen (Siemens Healthcare Diagnostics), with the BCS Classic assay. Dabigatran had no effect on plasminogen activity at any concentration.

Protein C

Protein C activity was measured in 3 laboratories using 3 different assays, 1 clot based (protein C clotting, Siemens Healthcare Diagnostics) and 2 chromogenic based (HemosIL protein C [Instrumentation Laboratories] and Stachrom protein C [Diagnostica Stago]). The clot-based method is aPTT based on the ability of APC inhibition of activated factors V and VIII. The chromogenic methods detect the activity of APC using a specific chromogenic substrate. Falsely elevated protein C activity, measured with clot-based methods, was evident in the presence of dabigatran at concentrations of approximately 150 ng/mL or higher **Figure 4**. The factitious increase in protein C activity is dose dependent, with results exceeding test linearity at doses of 400 to 500 ng/mL. As expected, chromogenic methods demonstrated no significant effect to varying concentrations of dabigatran.

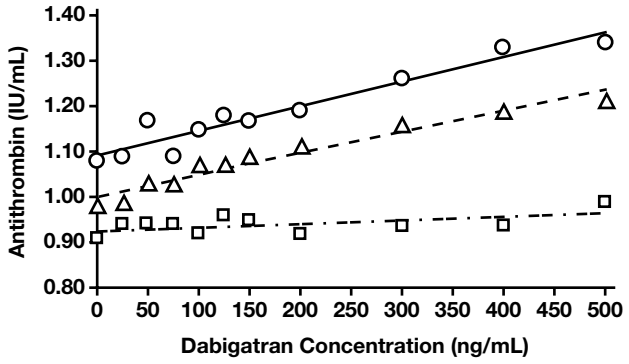

Figure 3 Effect of dabigatran on factor Xa– and factor IIa–based antithrombin activity assays. The effect of increasing concentrations of dabigatran mixed with normal plasma on antithrombin activity assays are shown. Assays based on inhibition of thrombin are indicated by both circles and triangles, and an assay based on factor Xa inhibition is indicated by squares, where 0 concentration dabigatran represents normal pooled plasma. The factor IIa–based assays therefore have the potential to falsely indicate a normal result in a patient with antithrombin deficiency.

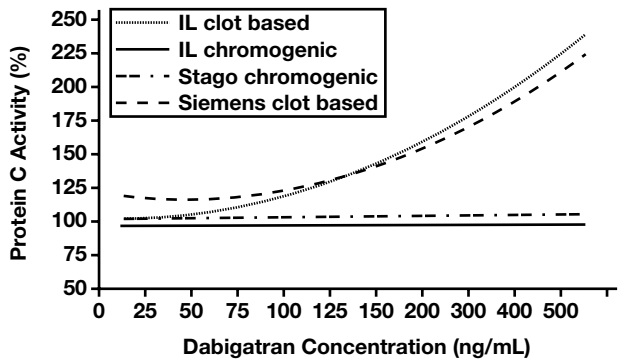

Figure 4 Effect of dabigatran on clot-based and chromogenic protein C activity assays. The effect of increasing concentrations of dabigatran mixed with normal plasma on clot-based or chromogenic protein C activity assays are shown. Both clot-based assays demonstrate increasing protein C activity with increasing concentrations of dabigatran while both chromogenic assays show essentially no effect. The clot-based assays therefore have the potential to falsely indicate a normal result in a patient with protein C deficiency. IL, Instrumentation Laboratories.

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Protein S

Protein S was measured in 3 laboratories using 2 clot-based assays (CRYOcheck CLOT S [Precision BioLogic] and Star STAclot-protein S [Diagnostica Stago]) on the BCS Classic instrument and 1 antigen-based free protein S antigen method (STA-Liatest free protein S [Diagnostica Stago]). Clot-based protein S activity assays, whether aPTT or Russell viper venom based, were exquisitely sensitive to dabigatran, with significantly elevated results evident even at doses of 25 ng/mL **Figure 5**. This low drug concentration resulted in doubling of the baseline concentration, and the results exceeded linearity at dabigatran concentrations of approximately 75 ng/mL. The free protein S antigen assay did not show any significant effect of dabigatran at varying concentrations.

APC Resistance Assay

One site performed the APC assay using COATEST APC Resistance V (Instrumentation Laboratories) on the MDAII instrument (Tcoag Ireland). This clot-based method is a modified aPTT test and uses factor V–deficient plasma. Increasing APC ratios are seen with increasing dabigatran concentration **Figure 6**.

Discussion

Dabigatran etexilate is a new oral anticoagulant agent gaining in popularity in part because its routine use does not require laboratory monitoring and it is not influenced by changes in diet. Adverse events reported in patients receiving dabigatran include bleeding, stroke, and venous thromboembolism. Because of its action as a direct thrombin inhibitor, dabigatran is known to interfere with routine coagulation assays. The purpose of our current study was to determine the effect of dabigatran on special coagulation assays that may be used to assess a patient with an acute bleeding or thrombotic event. Although routine and specialty coagulation assays are generally not recommended while patients are taking dabigatran, such testing may be required for acutely ill individuals presenting to the emergency department who may be poor historians (eg, altered mental status, stroke), for patients unable to provide medication history (eg, severe trauma), or for patients presenting to clinicians with limited access to medical records.

In assessing the bleeding patient, the PT and aPTT are standard practice, first-line assays. Subsequent testing algorithms often include mixing studies and factor activity or inhibitor titer assessment. We showed herein that relatively low levels of dabigatran (≥75 ng/mL, which is below the typical therapeutic range) will yield mixing study results consistent with an aPTT inhibitor, whereas relatively high levels of dabigatran (~200 ng/mL or the higher end of the therapeutic range) are needed to suggest results consistent with inhibitor effect in the PT assay.

aPTT-based factor assays, specifically factors VIII, IX, and XI activities, show significant dabigatran effect. At dabigatran concentrations of 200 to 300 ng/mL (higher end of the therapeutic range), factor VIII activity fell below 10 IU/dL;

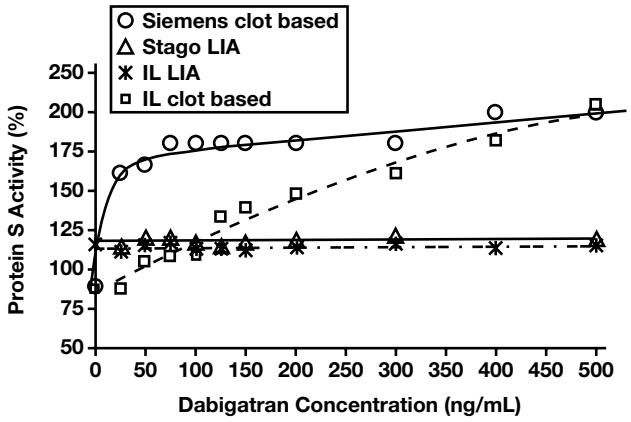

Figure 5 Effect of dabigatran on protein S activity and free protein S antigen assays. The effect of increasing concentrations of dabigatran mixed with normal plasma on protein S activity and free protein S antigen assays are shown. Both clot-based assays demonstrate increasing protein S activity with increasing concentrations of dabigatran, while both free protein S antigen assays show essentially no effect. The clot-based assays therefore have the potential to falsely indicate a normal result in a patient with protein S deficiency. IL, Instrumentation Laboratories; LIA, latex immunoassay.

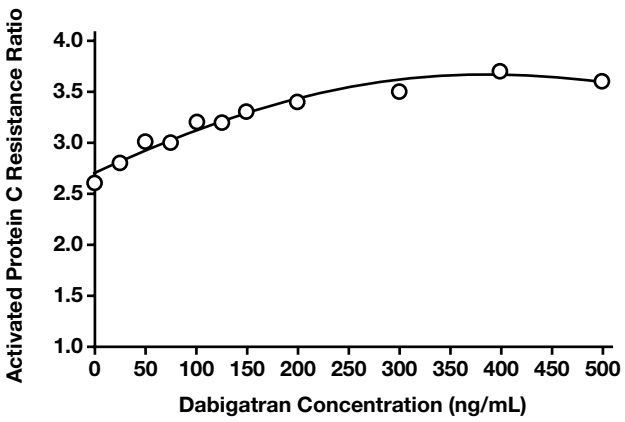

Figure 6 Effect of dabigatran on activated partial thromboplastin time (aPTT)–based activated protein C resistance assay. The effect of increasing concentrations of dabigatran mixed with normal plasma on the activated protein C resistance (APC) ratio using an aPTT-based method. The aPTT-based APC resistance assay demonstrate increasing ratio with increasing concentrations of dabigatran, with the potential to falsely indicate a normal ratio in a patient with the factor V Leiden mutation.

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normal plasma mixing studies showed lack of correction with time- and temperature-dependent prolongation; and the Bethesda titer was measurable. This effect is similar to that seen with other aPTT-based factor assays, including factor IX and factor XI activities. Intrinsic factor assays demonstrated nonparallelism when performed at 3 dilutions consistent with a nonspecific inhibitor effect. Increasing dilutions beyond the typical 1:40 did not result in progressively increasing activity, and as such the inhibitor effect could not be diluted to reveal the true underlying intrinsic factor activity. Therefore, aPTT-based screening tests, mixing studies, factor activity analysis, and even Bethesda assays may yield spurious results in the presence of dabigatran, leading to a false impression of either a factor deficiency or the presence of a specific factor inhibitor. PT-based factor assays, specifically factors II, V, and X, demonstrate significantly less dabigatran effect than aPTT-based factor measurements. Factor X showed a minimal decrease in activity only at the highest doses, whereas factor II demonstrated a drug-dependent inhibition of activity beginning at about 100 ng/mL of dabigatran so that at 500 ng/mL factor II activity was about 25% of the baseline value. PT-based factor assays did not demonstrate factitiously positive Bethesda titers in the presence of even high doses of dabigatran. The consequence for such misleading assessment, especially involving intrinsic factor analysis, would lead to the failure of conventional management to "correct" dabigatran coagulopathies.

Testing for antithrombin activity, protein C, protein S, APC resistance, and to a lesser degree, plasminogen is commonly used for assessing patients with thromboembolic complications. We demonstrated that clot-based methods are significantly affected by even small amounts of dabigatran, resulting in overestimation of protein C and protein S activities and a falsely elevated APC resistance ratio. The reptilase time, also a clot-based assay, was not affected by dabigatran as has been shown previously.[7] Reptilase is a snake venom that converts fibrinogen to fibrin through the cleavage of fibrinopeptide A. This clot-based assay is prolonged by some but not all antithrombins. A factor Xa–based antithrombin assay was unaffected by the presence of dabigatran, but a factor IIa–based assay overestimated the antithrombin concentration as previously reported.[7] Other testing platforms, such as select chromogenic or latex immunoassays including plasminogen activity, quantitative D-dimer, free protein S antigen, chromogenic protein C activity, von Willebrand factor antigen, and von Willebrand factor activity, are not affected by the presence of dabigatran. Laboratories with limited access to patient information (eg, reference laboratories) may require a mechanism for determining the suitability of all plasma samples, including those from patients treated with novel anticoagulants, for coagulation testing.

Potential weaknesses of our study include the fact that testing was performed using plasma samples enriched with dabigatran and not from patients currently receiving dabigatran treatment. Dabigatran directly inhibits both free and fibrin-bound thrombin. Therefore the observations would not be expected to differ if samples from patients taking dabigatran were compared with spiked samples because dabigatran was used and not the prodrug. Secondly, testing was performed at the discretion of each testing laboratory, and therefore not all possible methods for each assay were included. It is plausible that our observations may vary when other instrument reagent combinations are used for a given analyte. In this study we described the effects of different platforms, eg, clot based vs chromogenic, and the effect of dabigatran on the former but rarely on the latter. Further evaluation of the effect of this drug on other coagulation-related studies may be warranted, especially in those assays in which thrombin generation is required or when thrombin is a component of the assay.

In summary, dabigatran demonstrates an inhibitor effect in the hemostasis laboratory and shows clinically significant interference in various clot-based and factor IIa–based chromogenic assays **Table 3**. The presence of dabigatran can mimic a specific factor inhibitor, especially recapitulating a factor VIII inhibitor. Dabigatan can result in significant

Table 3
Effect of Dabigatran on Common Special Coagulation Assays

Assay	Dabigatran Effect on Test Results
APC resistance with factor V–deficient plasma	Factitiously elevated ratio
Antithrombin activity	
Factor Xa based	No effect
Factor IIa based	Factitiously overestimated
D-dimer	No effect
Factor assays	
aPTT based, 1 stage	Factitiously low factors VIII, IX, XI
PT based, 1 stage	Factitiously low factors II, V, VII, X[a]
Chromogenic factor VIII activity	No effect
Heparin (anti-Xa) assay (chromogenic method)	No effect
Inhibitor assay—aPTT-based Bethesda titer	Factitiously elevated at higher drug concentrations
Inhibitor screen (mixing studies)	
aPTT mixing study	Incomplete correction
PT mixing study	Incomplete correction
Plasminogen activity (chromogenic method)	No effect
Protein C activity	
aPTT clot based	Factitiously increased
Chromogenic	No effect
Protein S activity	
aPTT clot based	Factitiously increased
Free protein S antigen—LIA method	No effect
Reptilase time	No effect
von Willebrand factor	
Ristocetin cofactor (activity)	No effect
Antigen—LIA method	No effect

APC, activated protein C; aPTT, activated partial thromboplastin time; LIA, latex immunoassay; PT, prothrombin time.
[a] Effect occurs only at higher drug concentrations.

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overestimation of protein C and S activities when measured with a clot-based assay, mild overestimation of antithrombin activity when measured using a chromogenic IIa–based assay, and a factitiously elevated APC resistance ratio with an aPTT-based assay. These factitious coagulation test results seen in samples containing dabigatran can lead to inappropriate result interpretation, misdiagnosis, and patient mismanagement. Laboratories performing special coagulation assays should have access to information regarding a patient's anticoagulant medications or should develop means to identify the presence of the drug so that factitious results will not be reported.

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