Effect of Fondaparinux on Coagulation Assays

Results of College of American Pathologists Proficiency Testing

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• ***Context.*—Fondaparinux, a factor Xa inhibitor, is approved for thromboprophylaxis after orthopedic surgery and for treatment of venous thromboembolism. It may also be efficacious, safe, and cost-effective for other patients; thus, more widespread use of fondaparinux is likely. The effect of fondaparinux on coagulation testing needs to be thoroughly examined.**

***Objective.*—To report the effects of fondaparinux on coagulation tests (prothrombin time, activated partial thromboplastin time, fibrinogen, antithrombin, factor VIII, thrombin time, anti–factor Xa) across diverse methodologies.**

***Design.*—Samples with different concentrations of fondaparinux (0, 0.4, 0.8, and 2.0 μg/mL) were sent to laboratories participating in the College of American Pathologists Comprehensive Coagulation proficiency survey (N = 898). Laboratory-specific methods were used to assay coagulation parameters.**

***Results.*—Prophylactic or therapeutic fondaparinux prolonged the prothrombin time by approximately 1 second and the activated partial thromboplastin time by 4 to 5 seconds, and reduced factor VIII from 119% to 107% and 102%, respectively. Supratherapeutic fondaparinux reduced factor VIII to 85%. The activated partial thromboplastin time was prolonged in 19%, 29%, and 52% of laboratories with prophylactic, therapeutic, and supratherapeutic fondaparinux levels, respectively. Fibrinogen, antithrombin, and thrombin time assays did not show clinically significant changes. When measuring fondaparinux concentration using an anti–factor Xa assay, the most accurate results were obtained when fondaparinux was used as the calibrator.**

***Conclusions.*—Fondaparinux, even in prophylactic doses, slightly prolongs the prothrombin time and activated partial thromboplastin time and can interfere with factor VIII assays, but it has no clinically relevant effect on fibrinogen, antithrombin, or thrombin time. A fondaparinux standard curve should be used for reporting fondaparinux levels using an anti–factor Xa assay.**

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Fondaparinux is a synthetic factor Xa inhibitor based on the active site pentasaccharide of heparin. Based on 5 randomized double-blind trials, fondaparinux became the first synthetic factor Xa inhibitor to be approved for clinical use by the Food and Drug Administration specifically for thromboprophylaxis after orthopedic surgery.[1–5] More recently, a number of studies have assessed its efficacy in nonorthopedic surgical and medical patients. Fondaparinux has been shown to be equally as effective and safe as dalteparin in high-risk patients undergoing abdominal surgery.[6] In acutely ill medical patients, fondaparinux has significantly reduced the incidence of venous thromboembolism when compared with placebo.[7] Fondaparinux has already been approved for treatment of deep vein thrombosis as well as hemodynamically stable pulmonary embolism,[8,9] and it is being assessed as adjunct treatment in acute coronary syndromes.[10–12]

One of the potential advantages of fondaparinux in comparison to low-molecular-weight heparin (LMWH) is the prevention of heparin-induced thrombocytopenia. No cases of heparin-induced thrombocytopenia have been reported in any of the large phase 2 and 3 fondaparinux clinical studies. Consistent with this finding, fondaparinux did not interact with platelet factor 4 in vitro in several studies.[13–17] Although anti-PF4/heparin antibodies were found in patients receiving fondaparinux, they did not react with PF4/fondaparinux complexes, making them unlikely to cause heparin-induced thrombocytopenia during fondaparinux use.[18] There is also some evidence that fondaparinux might be efficacious and safe in treatment of heparin-induced thrombocytopenia,[19] although larger studies are necessary to assess this finding further.

Considering that fondaparinux might become more widely used, the effects of this drug on the coagulation parameters routinely tested in US laboratories have not been adequately assessed. The experience with patient samples from the original trial has shown that 2.8 mg fon-

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daparinux slightly elevated the prothrombin time (PT) by 1 second and activated partial thromboplastin time (aPTT) by 5 to 6 seconds, using a single reagent–instrument combination.[20] A recent study examined the effects of fondaparinux and direct thrombin inhibitors on coagulation testing. Using mostly Dade Behring reagents, the study found that fondaparinux affected protein S activity resulting in false elevations of protein S levels even at very low (0.2 μg/mL) drug concentrations.[21] Fondaparinux did not affect fibrinogen, protein C, antithrombin, plasminogen, von Willebrand factor, D-dimer, or factors II, IX, or X. To further assess the influence of fondaparinux on coagulation testing across a wide variety of reagents being used in the United States, we report the results of a College of Pathologists (CAP) proficiency test survey in which the laboratories were asked to perform PT, aPTT, fibrinogen, antithrombin, factor VIII, thrombin time, and anti–factor Xa assays in samples supplemented with different levels of fondaparinux.

MATERIALS AND METHODS

Proficiency test samples were prepared at bioMerieux Inc. starting with a pool of plasma from 20 healthy donors. Fondaparinux (Arixtra) was added to a final concentration of 0, 0.4, 0.8, or 2.0 μg/mL. The volume of anticoagulant added was less than 0.067% of the total volume, a trivial amount, in order to avoid a dilutional effect. Aliquots of all samples were lyophilized and sent to each laboratory participating in the 2004 CAP Comprehensive Coagulation Proficiency Test Survey CG2-B done under the auspices of the CAP Coagulation Resource Committee. The laboratories reconstituted the samples and performed the requested assays according to their standard operating procedures specific for each test. Methods and instruments used by the participants are summarized in Tables 1 through 7. Manufacturers included bioMerieux (Durham, NC), BBL/BioQuest (Cockeysville, Md), Chromogenix/Hemoliance/Instrumentation Laboratories (Lexington, Mass), Dade Behring (Deerfield, Ill), Diagnostica Stago (Parsippany, NJ), and Sigma (St Louis, Mo).

In addition to the reporting of quantitative results, a qualitative result for PT and aPTT measurements (normal vs abnormal) was reported by each laboratory based on the method and reference range valid for that laboratory. For the testing of fondaparinux concentration using anti–factor Xa assays, a standard curve constructed with fondaparinux was recommended. If the laboratory had not established a standard curve constructed with fondaparinux, they were asked to use their LMWH standard curve, and if a LMWH standard curve was also not available, they were asked to use an unfractionated heparin (UFH) standard curve.

To assess statistical significance of the results, all-method means for each concentration of fondaparinux were compared using the *t* test. In addition, within each individual method, the means of the different samples were compared using the *t* test. Results were deemed to be statistically significant if the *P* value was less than .05.

RESULTS

More than 1000 laboratories participated in the 2004 CAP Comprehensive Coagulation Survey (CG2-B) assessing the effect of fondaparinux on frequently assayed coagulation parameters. Analysis was performed when 80% of participants had responded (N = 898). The large array of methods used by the laboratories is summarized in Tables 1 through 7. The all-method means shown in the tables are weighted means. The samples studied included normal plasma lacking fondaparinux and normal plasma supplemented with fondaparinux to final concentrations of 0.4, 0.8, and 2.0 μg/mL. Based on previous clinical trials, plasma concentrations of 0.4 and 0.8 μg/mL correspond to plasma concentrations obtained in patients treated with a prophylactic dose and a therapeutic dose of fondaparinux, respectively.[11,22] A fondaparinux concentration of 2.0 μg/mL has been easily achieved in healthy elderly patients receiving >10 mg IV fondaparinux, but this level is considered supratherapeutic.[22]

The presence of fondaparinux had a statistically significant effect on the PT and aPTT measurements for all methods tested in the survey. Comparison of the means for each reagent–instrument combination shown in Table 1 shows that mean PT values in the samples with a prophylactic level of fondaparinux (0.4 μg/mL) were on average 0.9 seconds longer than in the samples without the drug (range, 0.53–1.4 s). The mean PT values in the samples with a therapeutic level of fondaparinux (0.8 μg/mL) were on average 1.2 seconds longer than in the samples without the drug (range, 0.70–1.8 s). The mean PT values in the samples with a supratherapeutic level of fondaparinux (2 μg/mL) were on average 1.8 seconds longer than in the samples without the drug (range, 1.1–2.9 s). Qualitative analysis of the data by the labs indicated that 2%, 3%, and 24% of participating laboratories reported an abnormal PT in the samples with prophylactic, therapeutic, and supratherapeutic fondaparinux levels, respectively. *P* values were calculated for each individual reagent–instrument combination that had N >10, and each was statistically significant (Table 1).

The effect of fondaparinux on the aPTT is shown in Table 2. In the sample with no fondaparinux, 2% of aPTT values were reported as abnormal. In the sample with prophylactic fondaparinux, 19% of aPTT values were abnormal. Therapeutic fondaparinux levels caused 29% of aPTTs to be reported as abnormal, and this number increased to 52% in samples with supratherapeutic fondaparinux. On average, mean aPTT values in the samples with a prophylactic level of fondaparinux (0.4 μg/mL) were 3.8 seconds longer than in the samples without the drug (range, 1–7 s). Mean aPTT values in the samples with a therapeutic level of fondaparinux (0.8 μg/mL) were on average 4.6 seconds longer than in the samples without the drug (range, 2–8 s). The mean aPTT values in the samples with a supratherapeutic level of fondaparinux (2 μg/mL) were on average 6.2 seconds longer than in the samples without the drug (range, 3–11 s). These data indicate that fondaparinux may have a clinically relevant prolongation effect on the aPTT. *P* values were calculated for each individual reagent–instrument combination that had N >10, and each was statistically significant (Table 2).

Results of factor VIII testing are shown in Table 3. All fondaparinux concentrations reduced the measured factor VIII, with the largest effect seen in samples with supratherapeutic fondaparinux levels. These data suggest that fondaparinux can interfere with factor VIII assays, even at prophylactic levels.

As shown in Tables 4, 5, and 6, fondaparinux had very little effect on antithrombin assays, fibrinogen assays, or thrombin time. Some of the changes in the means reported were statistically significant but did not appear to be clinically significant. Thrombin time results with the Dade-Sysmex reagent–instrument combination were different from those with the other reagent–instrument combinations (Table 6). This did not appear to be related to fondaparinux because results in an additional specimen that contained fondaparinux with elevated factor VIII were

Table 1. Prothrombin Time Measured in Samples With Different Fondaparinux Concentrations*

Fondaparinux, μg/mL	0.0	0.4	0.8	2.0
Reagent/Instrument	**Time, s (No. of Laboratories)**			
BioMerieux Simplastin HTF				
BioMerieux MDA-180	11.61 ± 0.38 (42)	12.61 ± 0.30 (42)	12.92 ± 0.29 (41)	13.75 ± 0.36 (42)
BioMerieux Coag-A-Mate MTX	11.2 (8)	12.4 (8)	12.6 (8)	13.3 (8)
BioMerieux Simplastin L				
BioMerieux MDA-180	11.36 ± 0.24 (23)	12.37 ± 0.22 (22)	12.57 ± 0.22 (23)	12.96 ± 0.29 (23)
BioMerieux Coag-A-Mate MTX	11.3 (8)	12.3 (8)	12.5 (8)	12.9 (8)
Dade Behring Innovin				
Dade Behring BCS	10.44 ± 0.23 (64)	11.00 ± 0.18 (65)	11.15 ± 0.20 (64)	11.53 ± 0.22 (65)
Dade Behring/Sysmex CA-Series	10.20 ± 0.31 (105)	10.83 ± 0.26 (106)	10.98 ± 0.27 (107)	11.41 ± 0.31 (106)
Diagnostica Stago STA Compact	9.3 (6)	10.2 (6)	10.3 (6)	10.8 (6)
Diagnostica Stago STA-R	9.5 (6)	10.2 (6)	10.5 (6)	10.9 (6)
Hemoliance ELECTRA 1400C, 1600C, 1800C	9.81 ± 0.38 (11)	10.34 ± 0.31 (12)	10.63 ± 0.39 (12)	10.99 ± 0.31 (12)
Dade Behring Thromboplastin C Plus				
Dade Behring BCS	10.84 ± 0.36 (23)	12.20 ± 0.27 (23)	12.60 ± 0.31 (23)	13.56 ± 0.37 (23)
Dade Behring/Sysmex CA-Series	10.52 ± 0.38 (55)	11.79 ± 0.39 (55)	12.14 ± 0.40 (56)	13.09 ± 0.50 (55)
Hemoliance ELECTRA 1400C, 1600C, 1800C	11.07 ± 0.31 (13)	12.11 ± 0.18 (14)	12.52 ± 0.18 (14)	13.41 ± 0.22 (14)
Dade Behring Thromborel S				
Dade Behring BCS	11.7 (6)	12.7 (6)	12.9 (6)	13.6 (6)
Dade Behring/Sysmex CA-Series	10.5 (6)	11.5 (5)	11.7 (5)	12.2 (5)
Diagnostica Stago Neoplastin CI, ISI 1.7-1.9				
Diagnostica Stago STA	12.09 ± 0.46 (16)	12.87 ± 0.33 (15)	13.25 ± 0.44 (16)	13.66 ± 0.47 (16)
Diagnostica Stago STA Compact	11.87 ± 0.39 (30)	13.01 ± 0.34 (31)	13.21 ± 0.40 (31)	13.69 ± 0.42 (31)
Diagnostica Stago STA-R	12.6 (7)	13.5 (7)	13.6 (7)	14.3 (7)
Diagnostica Stago Neoplastin CI Plus, ISI 1.3-1.5				
Diagnostica Stago STA	12.61 ± 0.39 (29)	13.43 ± 0.41 (30)	13.64 ± 0.41 (30)	14.08 ± 0.38 (30)
Diagnostica Stago STA Compact	12.64 ± 0.42 (146)	13.55 ± 0.39 (144)	13.77 ± 0.37 (144)	14.27 ± 0.44 (145)
Diagnostica Stago STA-R	13.07 ± 0.46 (47)	13.90 ± 0.43 (47)	14.14 ± 0.45 (47)	14.63 ± 0.47 (46)
Hemoliance Brain Thromboplastin				
Hemoliance ELECTRA 1400C, 1600C, 1800C	10.98 ± 0.28 (22)	12.25 ± 0.26 (22)	12.64 ± 0.30 (22)	13.67 ± 0.38 (22)
Hemoliance Recombiplastin				
Hemoliance ELECTRA 1400C, 1600C, 1800C	11.33 ± 0.41 (43)	12.27 ± 0.40 (42)	12.52 ± 0.40 (42)	13.36 ± 0.49 (43)
IL ACL Futura/Advance	9.7 (6)	10.7 (6)	11.1 (6)	11.8 (6)
IL Test PT-FIB HS				
IL ACL Futura/Advance	11.07 ± 0.35 (10)	12.47 ± 0.19 (10)	12.88 ± 0.26 (10)	13.95 ± 0.22 (10)
IL Test PT-FIB Recombinant				
IL ACL 9000	10.6 (6)	11.1 (6)	11.3 (6)	11.7 (6)
IL ACL Futura/Advance	9.51 ± 0.39 (19)	10.15 ± 0.50 (20)	10.23 ± 0.54 (20)	10.89 ± 0.49 (20)
IL Test PT-FIB, ISI 1.8-2.2				
IL ACL 9000	11.8 (5)	12.5 (5)	12.9 (5)	13.3 (5)
IL ACL, All Models Except 810, 9000, Futura/Advance	10.91 ± 0.44 (15)	12.15 ± 0.27 (15)	12.44 ± 0.29 (15)	13.15 ± 0.33 (15)
IL ACL Futura/Advance	10.59 ± 0.49 (27)	11.95 ± 0.32 (27)	12.25 ± 0.38 (27)	13.07 ± 0.44 (27)
Sigma Thrombomax HS				
Sigma-Amelung AMAX 190 Plus/200 (Mechanical)	12.99 ± 0.46 (11)	13.96 ± 0.48 (11)	14.24 ± 0.70 (11)	14.82 ± 0.82 (11)
All method mean	11.35 ± 1.06 (815)	12.26 ± 1.12 (816)	12.50 ± 1.14 (818)	13.10 ± 1.18 (819)
P value (vs no fondaparinux)		<.001	<.001	<.001

* Data are presented as the mean of prothrombin time measurements in seconds ± SD reported by the laboratories using a given reagent/instrument combination. For methods used by less than 10 laboratories, the median is given. For each reagent/instrument combination where N ≥10, the mean prothrombin time in the sample containing no fondaparinux was significantly lower ($P < .05$) then the mean in each of the corresponding samples containing fondaparinux.

similar to results in the specimen that did not contain fondaparinux (data not shown).

Fondaparinux, in general, does not require laboratory monitoring. Nevertheless, in special circumstances such as renal failure, low or high patient weight, or pregnancy or in the elderly, monitoring may be desired. The laboratories were asked to measure fondaparinux using the anti–factor Xa assay, using a fondaparinux, LMWH, or UFH standard

Table 2. Activated Partial Thromboplastin Time Measured in Samples With Different Fondaparinux Concentrations*

Fondaparinux, μg/mL	0.0	0.4	0.8	2.0
Reagent/Instrument	**Time, s (No. of Laboratories)**			
BioMerieux Platelin				
BioMerieux MDA-180	26.7 ± 0.9 (62)	32.0 ± 1.0 (63)	33.1 ± 1.0 (63)	35.0 ± 1.1 (63)
BioMerieux Coag-A-Mate MTX	26.9 ± 0.9 (16)	32.3 ± 0.8 (16)	33.3 ± 0.9 (16)	35.2 ± 1.0 (16)
Dade Behring Actin FSL				
Dade Behring BCS	31.4 ± 1.3 (69)	34.3 ± 1.2 (69)	34.9 ± 1.3 (70)	36.1 ± 1.4 (67)
Dade Behring/Sysmex CA-Series	31.4 ± 0.9 (119)	34.0 ± 1.0 (121)	34.5 ± 1.0 (119)	35.7 ± 1.2 (118)
Diagnostica Stago STA	31 (5)	33 (5)	33 (5)	34 (5)
Diagnostica Stago STA Compact	34 (6)	35 (6)	36 (6)	38 (6)
Hemoliance ELECTRA 1400C, 1600C, 1800C	32.2 ± 1.6 (11)	35.4 ± 1.4 (11)	36.2 ± 1.8 (11)	38.1 ± 2.3 (11)
Dade Behring Actin FS				
Dade Behring BCS	29.5 ± 1.0 (11)	32.1 ± 1.2 (12)	32.8 ± 1.1 (12)	33.7 ± 1.6 (12)
DADE/Sysmex CA-Series	29.0 ± 1.3 (31)	31.7 ± 1.6 (32)	31.8 ± 1.6 (32)	33.2 ± 1.9 (32)
Dade Behring Actin				
Dade Behring BCS	31 (6)	34 (6)	35 (6)	37 (6)
Dade Behring/Sysmex CA-Series	29.4 ± 3.3 (14)	33.8 ± 3.1 (15)	34.3 ± 2.9 (14)	36.3 ± 3.5 (14)
Hemoliance ELECTRA 1400C, 1600C, 1800C	27.0 ± 1.0 (13)	30.2 ± 1.1 (13)	30.9 ± 1.2 (13)	32.5 ± 0.8 (13)
Dade Behring Pathromtin SL				
Dade Behring BCS	35 (8)	42 (8)	43 (8)	46 (8)
Diagnostica Stago STA-PTT A				
Diagnostica Stago STA	29.3 ± 1.1 (45)	33.2 ± 1.1 (43)	34.1 ± 1.3 (44)	35.7 ± 1.3 (43)
Diagnostica Stago STA Compact	29.6 ± 1.1 (174)	34.0 ± 1.4 (172)	34.7 ± 1.3 (176)	36.5 ± 1.3 (172)
Diagnostica Stago STA-R	29.5 ± 0.9 (56)	33.2 ± 1.2 (56)	34.2 ± 1.1 (55)	35.8 ± 1.1 (55)
Hemoliance Synthasil				
Hemoliance ELECTRA 1400C, 1600C, 1800C	28.6 ± 0.7 (44)	32.8 ± 0.7 (43)	33.7 ± 0.9 (45)	35.1 ± 1.1 (44)
IL ACL 9000	30 (6)	34 (6)	35 (6)	36 (6)
IL ACL Futura/Advance	26.6 ± 0.8 (12)	31.5 ± 0.7 (12)	32.3 ± 1.0 (12)	33.6 ± 1.2 (12)
Hemoliance Thrombosil				
Hemoliance ELECTRA 1400C, 1600C, 1800C	28.2 ± 0.7 (16)	32.2 ± 0.8 (16)	32.9 ± 0.6 (16)	34.5 ± 0.7 (16)
IL Test APTT-SP				
IL ACL 9000	32 (9)	36 (9)	37 (9)	40 (9)
IL ACL, All Models Except 810, 9000, Futura/Advance	31.3 ± 2.0 (16)	35.8 ± 2.9 (16)	37.1 ± 1.7 (15)	39.7 ± 3.1 (16)
IL ACL Futura/Advance	29.6 ± 1.4 (48)	34.3 ± 1.7 (49)	35.4 ± 1.4 (50)	37.1 ± 1.9 (50)
Sigma APTT/Alexin LS				
Sigma-Amelung AMAX 190 Plus/200 (Mechanical)	31 (7)	34 (7)	35 (7)	37 (7)
Sigma APTT/Alexin				
Sigma-Amelung AMAX 190 Plus/200 (Mechanical)	31 (8)	35 (8)	35 (8)	36 (8)
All method mean	29.8 ± 1.6 (812)	33.6 ± 1.3 (814)	34.4 ± 1.4 (818)	36.0 ± 1.6 (809)
P value (vs no fondaparinux)		<.001	<.001	<.001

* Data are presented as the mean of activated partial thromboplastin time in seconds ± SD reported by the laboratories using a given reagent/instrument combination. The number of laboratories performing each test is indicated in parentheses. For testing performed by less than 10 laboratories, the median is given. For each reagent/instrument combination where N ≥10, the mean activated partial thromboplastin time in the sample containing no fondaparinux was statistically significantly lower ($P < .05$) than the mean in each of the corresponding samples containing fondaparinux.

curve, depending on which curve was currently in use by the laboratory. As shown in Table 7, using fondaparinux to make a standard curve gave very accurate results for fondaparinux levels in all samples tested. The LMWH standard curve allowed only for an approximation of the fondaparinux level in the sample, whereas UFH gave unreliable results.

COMMENT

This study was designed to measure the effect of fondaparinux on frequently assessed coagulation parameters. Fondaparinux is not metabolized by the liver, and it is excreted in the urine in its fully active form.[20,22] To approximate the prophylactic, therapeutic, and supratherapeutic levels of fondaparinux, this study used samples of normal plasma spiked with different levels of fondaparinux. Participation of a vast number of laboratories guaranteed that an array of instruments and reagents would be used. Fondaparinux had the most pronounced effect on aPTT measurements. Even prophylactic levels of fondaparinux prolonged the aPTT by 3.8 seconds on average. This increased to 4.6 seconds and 6.2 seconds in the samples with therapeutic and supratherapeutic levels of fondaparinux, respectively. In addition to furnishing the nu-

Table 3. Factor VIII Level Measured in Samples With Different Fondaparinux Concentrations*

Fondaparinux Concentration, μg/mL	0.0	0.4	0.8	2.0
Reagent/Instrument	**Mean Factor VIII, % (No. of Laboratories)**			
BioMerieux Platelin L				
BioMerieux MDA-180	106.6 ± 11.3 (53)	97.7 ± 8.6 (52)	94.8 ± 11.2 (52)	79.6 ± 10.4 (49)
BioMerieux Coagmate MTX	118 (7)	100 (7)	92 (7)	74 (7)
Dade Behring Actin FSL				
Dade Behring BCS	116.1 ± 12.9 (50)	106.7 ± 12.2 (50)	101.7 ± 13.3 (51)	85.2 ± 12.7 (51)
Dade Behring/Sysmex CA-Series	122.0 ± 11.0 (43)	112.4 ± 10.0 (45)	108.0 ± 10.1 (42)	96.5 ± 14.9 (43)
Dade Behring Actin FS				
Dade Behring BCS	110 (9)	101 (9)	100 (8)	87 (9)
Dade Behring/Sysmex CA-Series	133.8 ± 19.0 (16)	125.7 ± 21.7 (16)†	122.3 ± 25.3 (16)†	103.4 ± 18.3 (16)
Dade Behring Actin				
Dade Behring/Sysmex CA-Series	114 (8)	112 (8)	103 (9)	88 (9)
Hemoliance ELECTRA 1400C, 1600C, 1800C	121 (9)	106 (9)	98 (9)	83 (9)
Dade Behring Pathromtin SL				
Dade Behring BCS	117 (8)	113 (9)	109 (9)	95 (9)
Diagnostica Stago STA-PTT A				
Diagnostica Stago STA	125.1 ± 15.4 (43)	111.3 ± 11.4 (43)	107.5 ± 13.9 (44)	87.4 ± 12.7 (38)
Diagnostica Stago STA Compact	120.5 ± 16.1 (79)	109.0 ± 15.6 (79)	100.7 ± 15.3 (79)	80.2 ± 13.9 (75)
Diagnostica Stago STA-R	128.6 ± 16.4 (39)	115.6 ± 15.1 (39)	110.3 ± 17.1 (39)	90.2 ± 14.7 (38)
Hemoliance Synthasil				
Hemoliance ELECTRA 1400C, 1600C, 1800C	138.3 ± 23.4 (16)	114.8 ± 19.9 (16)	104.3 ± 17.6 (16)	86.6 ± 26.6 (16)
Hemoliance Thrombosil				
Hemoliance ELECTRA 1400C, 1600C, 1800C	124.7 ± 18.7 (10)	108.8 ± 15.4 (10)	103.1 ± 17.1 (10)	92.1 ± 12.9 (10)
IL Test APTT-SP				
IL ACL, ALL Models Except 810, 9000, Futura/Advance	107.2 ± 19.3 (10)	87 (9)	80 (9)	66 (9)
IL ACL Futura/Advance	111.5 ± 14.8 (30)	100.1 ± 10.4 (27)	97.4 ± 12.4 (28)	83.3 ± 11.9 (27)
All method mean	118.6 ± 17.2 (530)	106.9 ± 15.4 (529)	101.5 ± 16.8 (529)	85.0 ± 15.9 (516)
P value (vs. no fondaparinux)		<.001	<.001	<.001

* Data are presented as the mean factor VIII level (percent of normal) ± SD reported by the laboratories using a given reagent/instrument combination. The number of laboratories performing each test is indicated in parentheses. For testing performed by fewer than 10 laboratories, the median is given. For each reagent/instrument combination where N ≥10, the mean factor VIII in the sample containing no fondaparinux was statistically significantly different ($P < .05$) than the mean in each of the corresponding samples containing fondaparinux, except as noted in the table.

† The mean factor VIII level in this sample is not statisitically different from the mean factor VIII level in the sample with no fondaparinux.

Table 4. Antithrombin Level Measured in Samples With Different Fondaparinux Concentrations*

Fondaparinux Concentration, μg/mL	0.0	0.4	0.8	2.0
Assay	**Mean Antothrombin, % (No. of Laboratories)**			
Functional antithrombin assays				
IIa-based assays				
BioMerieux Chromostrate (IIa based)	89.5 ± 8.1 (15)	94.0 ± 11.3 (16)	92.1 ± 10.3 (15)	93.2 ± 8.3 (15)
Dade Behring Berichrom (IIa based)	97.7 ± 6.3 (100)	100.4 ± 5.7 (100)†	100.6 ± 6.1 (98)†	99.3 ± 6.3 (100)
Diagnostica Stago Stachrom (IIa based)	102.9 ± 7.6 (145)	104.0 ± 6.8 (147)	104.3 ± 6.2 (145)	103.2 ± 6.9 (146)
IIa-based all method mean	100.1 ± 3.6 (260)	102.0 ± 2.7 (263)	102.2 ± 3.1 (258)	102.1 ± 2.7 (263)
Xa-Based Assays				
Chromogenix Coamatic (Xa based)	96.9 ± 9.1 (10)	99.7 ± 7.0 (10)	100.2 ± 6.8 (10)	97.1 ± 8.5 (10)
Hemoliance Electrachrome (Xa based)	94.9 ± 6.3 (11)	95.1 ± 6.3 (11)	95.5 ± 6.2 (11)	97.5 ± 6.2 (11)
IL Test (Xa based)	97.6 ± 6.2 (47)	100.2 ± 7.0 (46)	100.0 ± 7.4 (47)	100.3 ± 5.8 (47)†
Xa-based all method mean	97.1 ± 1.0 (68)	99.3 ± 1.9 (67)	99.3 ± 1.7 (68)	99.4 ± 1.4 (68)
Antigenic antithrombin assay				
Diagnostica Stago Liatest	101.1 ± 7.5 (14)	101.4 ± 7.6 (14)	102.4 ± 5.3 (14)	98.6 ± 11.9 (15)

* Data are presented as the mean of antithrombin (percent of normal) ± SD reported by the laboratories using a given assay. The number of laboratories performing each test is indicated in parentheses. For each method listed, there was no significant difference among the difference among the different specimens, except as noted in the table.

† The mean antithrombin level in this sample is statistically significantly different ($P < .05$) from the mean antithrombin level in the sample without any fondaparinux, but the difference is not clinically significant.

Table 5. Fibrinogen Measured in Samples With Different Fondaparinux Concentrations*

Fondaparinux Concentration, μg/mL	0.0	0.4	0.8	2.0
Method Type	**Mean Fibrinogen, % (No. of Laboratories)**			
Prothrombin time-based fibrinogen assays	286.7 ± 25.1 (101)	296.5 ± 30.0 (102)	297.1 ± 31.2 (102)	299.7 ± 38.2 (102)
Optical detection assays	259.1 ± 26.5 (551)	263.4 ± 28.5 (553)	265.2 ± 28.5 (554)	263.1 ± 30.9 (549)
Mechanical assays	269.3 ± 17.4 (314)	273.4 ± 16.5 (314)	273.7 ± 16.7 (316)	271.0 ± 16.6 (315)†

* Data are presented as the mean of fibrinogen (mg/dL) ± SD reported by the laboratories using a given type of assay. The number of laboratories performing each test is indicated in parentheses. For each method listed, the mean fibrinogen in the sample containing no fondaparinux was statistically significantly different ($P < .05$) than the mean in each of the corresponding samples containing fondaparinux, except as noted in the table, but the differences are not clinically significant.

† The mean fibrinogen level in this sample is not statistically significantly different from the mean fibrinogen level in the sample with no fondaparinux.

Table 6. Thrombin Time Measured in Samples With Different Fondaparinux Concentrations*

Fondaparinux Concentration, μg/mL	0.0	0.4	0.8	2.0
Instrument	**Time, s (No. of Laboratories)**			
BBL (BioQuest) Fibrometer	14.53 ± 3.57 (36)	14.60 ± 3.60 (37)	14.84 ± 3.75 (37)	14.84 ± 3.94 (37)
BioMerieux MDA-180	13.27 ± 1.21 (45)	13.37 ± 0.99 (44)	13.50 ± 1.26 (45)	13.39 ± 0.80 (44)
BioMerieux Coagmate MTX	12.75 ± 0.63 (11)	12.92 ± 0.67 (11)	13.04 ± 0.75 (11)	13.19 ± 0.79 (11)
Dade Behring BCS	17.64 ± 1.09 (56)	17.98 ± 0.99 (56)	18.07 ± 0.99 (56)†	18.18 ± 1.06 (57)†
Dade Behring/Sysmex CA-Series	28.30 ± 9.79 (70)	16.77 ± 3.22 (68)†	16.51 ± 3.19 (68)	16.15 ± 3.12 (70)
Diagnostica Stago ST4, Start 4, Start 8	16.18 ± 2.97 (15)	16.62 ± 3.32 (15)	17.55 ± 4.07 (15)	16.97 ± 3.56 (15)
Diagnostica Stago STA	15.58 ± 0.61 (44)	15.88 ± 0.67 (44)†	16.15 ± 0.74 (44)†	16.24 ± 0.89 (44)†
Diagnostica Stago STA Compact	15.80 ± 0.56 (120)	16.15 ± 0.58 (118)†	16.23 ± 0.60 (120)†	16.28 ± 0.54 (120)†
Diagnostica Stago STA-R	16.00 ± 0.57 (46)	16.37 ± 0.65 (45)†	16.48 ± 0.65 (46)†	16.56 ± 0.54 (46)†
Hemoliance ELECTRA 1400C, 1600C, 1800C	13.02 ± 3.50 (24)	13.53 ± 3.47 (25)	13.44 ± 3.52 (25)	13.78 ± 3.75 (25)
IL ACL 9000	18.96 ± 6.25 (10)	19.76 ± 6.97 (10)	20.02 ± 6.86 (10)	20.59 ± 7.52 (10)
IL ACL, All Models Except 810, 9000, Futura/Advance	19.01 ± 1.00 (11)	19.55 ± 1.11 (11)	18.54 ± 4.13 (13)	20.14 ± 1.17 (11)†
IL ACL Futura/Advance	18.72 ± 5.45 (40)	18.70 ± 4.84 (39)	19.21 ± 5.72 (40)	19.93 ± 5.69 (40)
Sigma-Amelung AMAX 190 Plus/200 (Mechanical)	14.89 ± 3.40 (15)	15.20 ± 3.41 (15)	15.45 ± 3.32 (15)	15.95 ± 3.35 (15)
Tilt Tube	18.2 (5)	18.2 (5)	18.0 (5)	18.0 (5)
All method mean	17.5 ± 4.5 (548)	16.2 ± 1.7 (543)	16.3 ± 1.0 (550)	16.4 ± 1.8 (543)

* Data are presented as the mean of thrombin time in seconds ± SD reported by the laboratories using an indicated instrument. The number of laboratories performed each test is indicated in parentheses. For testing performed by fewer than 10 laboratories, the median is given. For each instrument there was no significant difference among the different specimens, except as noted in the table.

† The mean thrombin time in this sample is statistically significantly different ($P < .05$) from the mean thrombin time in the sample without any fondaparinux, but the difference is not clinically significant.

Table 7. Anti–Factor Xa Assay in Samples With Different Fondaparinux Concentrations, μg/mL or U/mL*

Fondaparinux Concentration, μg/mL	Method/Reagent						
	Diagnostica Stago Rotachrom		Diagnostica Stago STA		Other Method		
	Standard Curve		Standard Curve		Standard Curve		
	LMWH	UFH	Fondaparinux	LMWH	Fondaparinux	LMWH	UFH
0	0.050 ± 0.046 (76)	0.028 ± 0.042 (22)	0.00 (6)	0.037 ± 0.039 (20)	NR	0.027 ± 0.043 (71)	0.028 ± 0.038 (12)
0.4	0.558 ± 0.076 (94)	0.645 ± 0.051 (24)	0.39 (7)	0.472 ± 0.074 (25)	NR	0.404 ± 0.145 (92)	0.172 ± 0.081 (13)
0.8	1.102 ± 0.084 (92)	1.003 ± 0.190 (25)	0.81 (7)	0.866 ± 0.135 (25)	0.79 (5)	0.716 ± 0.200 (90)	0.429 ± 0.148 (14)
2	2.138 ± 0.390 (81)	1.525 ± 0.604 (22)	2.10 (7)	1.729 ± 0.279 (22)	NR	1.427 ± 0.449 (87)	0.774 ± 0.388 (14)

* Units are μg/mL if fondaparinux was used for the standard curve preparation or U/mL if low-molecular-weight heparin or unfractionated heparin was used for the standard curve. The number of laboratories that performed each test is indicated in parentheses. LMWH indicates low-molecular-weight heparin; UFH, unfractionated heparin; and NR, not reported by College of American Pathologists when N <5. Data are presented as the mean of fondaparinux concentrations (except when testing was performed by less than 10 laboratories, the median is given).

merical data, the laboratories were also asked to classify the results as normal or abnormal based on the laboratory's reference ranges. Strikingly, 19% of aPTT values were reported as abnormal in samples with prophylactic fondaparinux and 29% in samples with therapeutic drug levels. Knowledge that fondaparinux can elevate the aPTT into an abnormal range is essential to preclude additional unnecessary laboratory evaluation or administration of blood products in patients on fondaparinux. An additional sample included in the CAP Survey contained prophylactic fondaparinux (0.4 μg/mL) with an elevated factor VIII level of 300% (data not shown). In this specimen, the aPTT

normalized or fell even lower than normal. This is explained by the fact that elevated factor VIII shortens the aPTT[23] and thus counteracts the influence of fondaparinux in the assay. High levels of factor VIII occur commonly in patients (due to acute phase reactions). Therefore, even though there is a clear correlation between the fondaparinux level and the increase in the aPTT value, these findings indicate that the aPTT should not be used for monitoring fondaparinux.

The influence of fondaparinux on the PT was less pronounced than on the aPTT. Only the sample with supratherapeutic fondaparinux was classified as having an abnormal PT value by a significant number of laboratories (24%). It remains to be seen whether the presence of other anticoagulants (for example, warfarin) would synergize with fondaparinux to affect the PT and international normalized ratio values.

Fondaparinux interfered with factor VIII assays, lowering the measured level of factor VIII in samples containing the anticoagulant. As factor VIII assays are aPTT based, the interference is apparently due to the ability of fondaparinux to prolong the aPTT. Although there were some changes of fibrinogen, thrombin time, and antithrombin measurements between samples, they were slight and would have no influence on patient management. Commercially available antithrombin functional assays are based on inhibition of either factor IIa or factor Xa. Since fondaparinux is a specific factor Xa inhibitor, it might be expected to exert an inhibitory effect on the antithrombin assays that are factor Xa based, falsely elevating the results. However, based on the results of this study, it appears that anti–factor Xa based assays are not more prone to the inhibitory effect of fondaparinux. This may be because factor Xa- (and factor IIa-) based assays supply heparin in the assay. Fondaparinux, a factor Xa inhibitor, would not be expected to inhibit thrombin (factor IIa) in the thrombin time assay or in the Clauss or Ellis methods of fibrinogen assays.

Finally, this study examined the possibility of using LMWH or UFH standard curves to assess the level of fondaparinux. A notable finding is that very few laboratories used fondaparinux to form the standard curves to measure fondaparinux levels. These few laboratories, however, reported impressively accurate results, confirming that this is the preferred method for fondaparinux measurement. Results obtained using a LMWH standard curve were less accurate. UFH was inaccurate and should not be used for reporting of fondaparinux levels.

The studies presented here have used normal plasma spiked with fondaparinux in vitro. This is appropriate since fondaparinux is not metabolized in the body. However, future studies to assess the in vivo influence of fondaparinux on coagulation parameters might be useful. This might be especially interesting in patients with venous thromboembolic events who are being switched to an oral anticoagulant.

In conclusion, fondaparinux can affect the PT and aPTT, even with prophylactic dosing. Fondaparinux may interfere with factor VIII assays. No clinically significant effect was seen with fibrinogen, thrombin time, or antithrombin assays. To measure fondaparinux with anti–factor Xa assays, fondaparinux should be used to construct the standard curve.

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