Original Article

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The Effect of Polybrene on an aPTT-based Lupus Anticoagulant Test in the Plasma of Patients Receiving Unfractionated Heparin

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Abstract:

Objective: Heparins can interfere with lupus anticoagulant (LA) testing, especially when the detection assays do not contain a heparin neutralizer. In this study we evaluated whether hexadimethrine bromide commonly known as polybrene was able to neutralize the effects of unfractionated heparin (UFH) on activated partial thromboplastin time (aPTT)-based LA determination.

Material and Methods: The influence of polybrene on the aPTT-based LA testing results of 14 patients receiving UFH therapy was studied. Measurements were performed using Dade Actin FSL (aPTT-LA screen) and Dade Actin FS (aPTT-LA confirm) reagents on a Sysmex CS-1600 analyzer. The aPTT-LA screen/confirm ratios before and after the polybrene treatments were compared.

Results: The UFH treatment affected the aPTT-LA screen/confirm ratio in four patterns, 1) no coagulation (NC code), 2) false positive, 3) false low, and 4) unchanged interpretation. After adding polybrene in the patient plasmas, the aPTT-LA screen/confirm ratios returned nearly to baseline without false positive or negative results.

Conclusion: The best practice for LA testing should be done outside anticoagulant therapies, but if necessary, the tests can be performed by adding the proper concentration of polybrene.

Keywords: aPTT, lupus anticoagulant, neutralization, polybrene, unfractionated heparin

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Introduction

Unfractionated heparin (UFH) is a medication used for the prevention and treatment of thrombosis. To reduce coagulation, UFH binds to antithrombin (AT) via a specific pentasaccharide sequence. This binding induces conformational changes in the AT molecule leading to increased anticoagulant activity of AT. Then the AT suppresses coagulation by inactivating thrombin and factor Xa.[1,2] The activated partial thromboplastin time (aPTT) ratio is a principal laboratory test used to monitor intravenous UFH therapy.[3] The generally accepted therapeutic range of UFH is 1.5–2.5 times the control when monitoring aPTT ratios, and 0.3–0.7 U/mL while monitoring by anti-factor Xa.[4–6] Previous studies demonstrated that an aPTT ratio of 1.5 to 2.5 times corresponded to a heparin level of 0.2 to 0.4 U/mL using the protamine titration heparin assay.[7,8]

Lupus anticoagulant (LA) testing is frequently ordered when a patient has an unexplained thrombotic event, thrombosis with an autoimmune disease, recurrent miscarriages, or as a follow-up to a prolonged aPTT. Patients with thrombosis require immediate and prolonged anticoagulation therapy. The use of anticoagulant drugs, especially UFH, frequently interferes with the results and interpretation of LA testing. However, there are various opinions about how to deal with this problem.[9–11] The guideline from the International Society on Thrombosis and Haemostasis (ISTH) 2020[11] recommends measuring anti-factor Xa activity together with LA testing in patients who are known to be on UFH, while the guideline from the British Committee for Standards in Haematology (BCSH) 2012[12] recommends not performing LA testing if the patient is receiving therapeutic doses of UFH, and the guideline from the Clinical and Laboratory Standards Institute (CLSI) 2014[13] suggests that LA can be detected in some cases where a heparin neutralizer is effective. As a result of these inconsistent guidelines, in the best practice, LA testing in patients receiving UFH would be postponed until anticoagulation has been discontinued for a suitable period of time. However, LA testing in patients receiving UFH is regularly requested.[10] A study in 18,676 LA test results found that the prevalence of heparin in samples submitted for LA testing was 11.0%.[14]

As a problem of antibody heterogeneity, no single assay can detect all LAs. Therefore, all three guidelines recommend to use two assays for LA testing, the Diluted Russell Viper Venom Time (dRVVT) for its specificity and the aPTT-based LA for its sensitivity.[15] The UFH may cause false positive results for LA testing, including prolonged aPTT and dRVVT.[9] As a result, anti-heparin agents are commonly added to commercial dRVVT kits to neutralize the effect of heparin. However, aPTTs are commonly used to monitor heparin, and the cost of heparin neutralizer is rather expensive when compared with the cost of aPTT testing. Therefore, many aPTT commercial kits do not contain anti-heparin agents.

Previous studies found that the much cheaper basic compounds, such as protamine sulfate and polybrene, could neutralize heparin, which is an acidic glycosaminoglycan.[16,17] One of these studies reported that protamine and polybrene could reverse heparin-induced anticoagulation after cardiopulmonary bypass in vitro, however, polybrene had greater potency in neutralizing high doses of heparin, and had less effect on coagulation tests.[17] Another study reported that polybrene was more stable in plasma than protamine.[18] Polybrene is a synthetic polymeric quaternary ammonium salt (a polycation polymer) which can neutralize the negatively charged heparin by forming inactive complexes.[16,18] It is one of the main compounds used in the dRVVT reagents.

In this study, we evaluated using polybrene to neutralize the effects of UFH on aPTT-based LA testing.

Material and Methods

Sample

This study was done following approval from the Ethics Review Board of the Faculty of Medicine of Prince of Songkla University, Thailand (REC. 61-189-5-2).

To assess the influences of UFH and polybrene on LA interpretation, the aPTT-LA screen/confirm ratios were determined in plasma samples collected from the residual plasmas sent for routine UFH dose monitoring by aPTT ratio assay in the Hematology Unit of the Pathology Department in Songklanagarind Hospital. As the guidelines recommend not to test LA during treatment with UFH, we do not normally have incident rates of uninterpreted results including false positives and no coagulation of aPTT-LA in the patient. Therefore, we did a preliminary study by testing aPTT-LA in the plasma of 10 patients who were being treated with UFH. The results from 5 of the 10 patients (50%) showed uninterpreted results. As a result, the sample size of this study was calculated by using the two dependent proportions formula as below:

$$n = \left[\frac{z_{1-\frac{\alpha}{2}} \sqrt{p_{01}+p_{10}} + z_{1-\beta} \sqrt{p_{01}+p_{10}-(p_{01}-p_{10})^2}}{\Delta} \right]^2$$

Proportion of pre-treatment with polybrene (p_{10}) = 0.500

Expected proportion of post-treatment with polybrene (p_{01})=0.000

Alpha (α)=0.05, Z(0.975)=1.959964

Beta (β)=0.20, Z(0.800)=0.841621

Sample size (n)=14

The samples of fourteen patients were collected at three random time points during UFH monitoring. All plasma samples were prepared from blood collected in 2.7-mL evacuated tubes (Vacutainer, Becton, Dickinson and Company Ltd, Oxford, England) containing 0.3 mL of 3.2% sodium citrate for a 9:1 ratio of blood to anticoagulant. The plasma samples were separated by double centrifugation at 2,000 g for 15 minutes, and the experiments were performed within 1 hour.

Lupus anticoagulant test and instrument

The aPTT-based LA assays were measured with Dade Actin FSL (aPTT-LA screen) and Dade Actin FS (aPTT-LA confirm) reagents (Dade Behring, Marburg, Germany). The principle of the assay is based on interference with an in vitro phospholipid-dependent coagulation test by LA which is an autoantibody that binds to a negatively charged phospholipid-binding protein. The aPTT-LA screen assay is an aPTT that uses a reagent with a minimal phospholipid concentration which is sensitive to LA, while the aPTT-LA confirm is an aPTT that uses a reagent with a high phospholipid concentration which is insensitive to LA. Therefore, a positive for LA is diagnosed whenever the aPTT-LA screen/confirm ratio is longer than the upper limit of a normal aPTT-LA screen/confirm ratio. The normal ranges of aPTT-LA screen, aPTT-LA confirm and aPTT-LA screen/confirm ratio were measured on plasma from 40 healthy donors. The mean±2 standard deviation (S.D.) for the aPTT-LA screen, aPTT-LA confirm and aPTT-LA screen/confirm ratios were 24.74–33.92, 21.41-30.45 seconds and 0.98-1.28, respectively. The LA positive cutoff value of the aPTT-LA screen/confirm ratio was >1.31. The normal range for aPTT-LA screen/confirm ratio was derived from the 99th percentile of the distribution of the tests. The LA examinations were performed on a CS-1600 instrument (Sysmex, Kobe, Japan). The upper limit of aPTT detection by this machine was 180 seconds, and the NC code would be shown when the clotting time was longer than 180 seconds.

To determine the effects of UFH and polybrene on the aPTT-based LA testing

Six doses of UFH (Heparin Leo, LEO Pharma A/S, Ballerup, Denmark) were used to simulate different

concentrations of heparin interference in the blood samples. Heparin (5,000 U/mL) was diluted in 0.15 M NaCl to obtain working solutions of 10, 20, 30, 40, 50 and 60 U/mL, then 4 µL of these solutions was added to 400 µL of plasma to obtain final concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 U/mL, respectively.

Polybrene (Sigma-Aldrich, Milwaukee, Wisconsin) was diluted to 10,000 µg/mL in 0.15M NaCl to prepare the stock solution, which was subsequently diluted with 0.15M NaCl to generate working solutions. Six concentrations of 150, 200, 250, 300, 350 and 400 µg/mL were prepared. With 40 µL of each working solution added to 400 µL of plasma by the analyzer, the final concentrations of polybrene in plasma were 15, 20, 25, 30, 35 and 40 µg/mL, respectively.

The aPTT-LA screen, aPTT-LA confirm and aPTT-LA screen/confirm ratio of pooled normal plasma (Control plasma N, Siemens, Marburg, Germany) treated with the six doses of UFH or the six doses of polybrene were determined in triplicate.

Determining the most effective concentration of polybrene for UFH neutralization in pooled normal plasma measured by aPTT-based LA assays

Pooled normal plasma was mixed with UFH for final concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 U/mL. Then, the solutions were treated with 15, 20, 25, 30, 35 and 40 µg/mL final concentrations of polybrene, respectively. The aPTT-LA screen, aPTT-LA confirm and aPTT-LA screen/confirm ratios were measured in each sample. Pooled normal plasma mixed with 44 µL of 0.15M NaCl was used for baseline values. The most effective concentration of polybrene for UFH neutralization was the minimum concentration that gave the least mean difference when compared to the baseline. The UFH levels were monitored by two assays, aPTT ratio and anti-factor Xa activity, within 1 hour using Actin FS (Dade Behring, Marburg, Germany) and a Berichrom Heparin chromogenic assay kit (Dade Behring, Marburg, Germany), respectively. All tests were performed in triplicate.

Determining the effect of UFH and polybrene on aPTT-based LA testing in plasma of patients receiving UFH The most effective concentrations of polybrene as determined from the earlier test results were used to neutralize various concentrations of UFH in plasma samples of 14 patients. All samples were from patients with no history of positive LA testing before the UFH therapy. The aPTT-LA screen/confirm ratio with or without polybrene were determined at three time-points after receiving UFH. The concentration of polybrene for UFH neutralization at each time point was selected following the UFH-monitoring aPTT ratio of the patients. The LA results after UFH neutralization at the three time-points were compared.

Statistical analysis

The analytical results are presented as means± S.D. Student's t test was used to calculate statistical significance compared to the baseline. The mean differences from baseline are presented with 95% confidence intervals. A p-value less than 0.05 was considered statistically significant.

Results

The effects of UFH on aPTT-based LA testing in pooled normal plasma

The aPTT-LA screen and aPTT-LA confirm values were markedly increased after treatment with UFH in a dose-dependent manner (Figure 1A). The NC code (indicating a clotting time >180 seconds) showed in all aPTT-LA confirm results in all treatments with UFH ≥0.5 U/mL. The aPTT-LA screen/confirm ratios became markedly lower from 1.03±0.005 to 0.94±0.010, 0.80±0.004, 0.77±0.009, 0.60±0.016, <0.48, and <0.46, when higher concentrations of UFH from 0 to 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6, respectively, were added (Figure 1B).

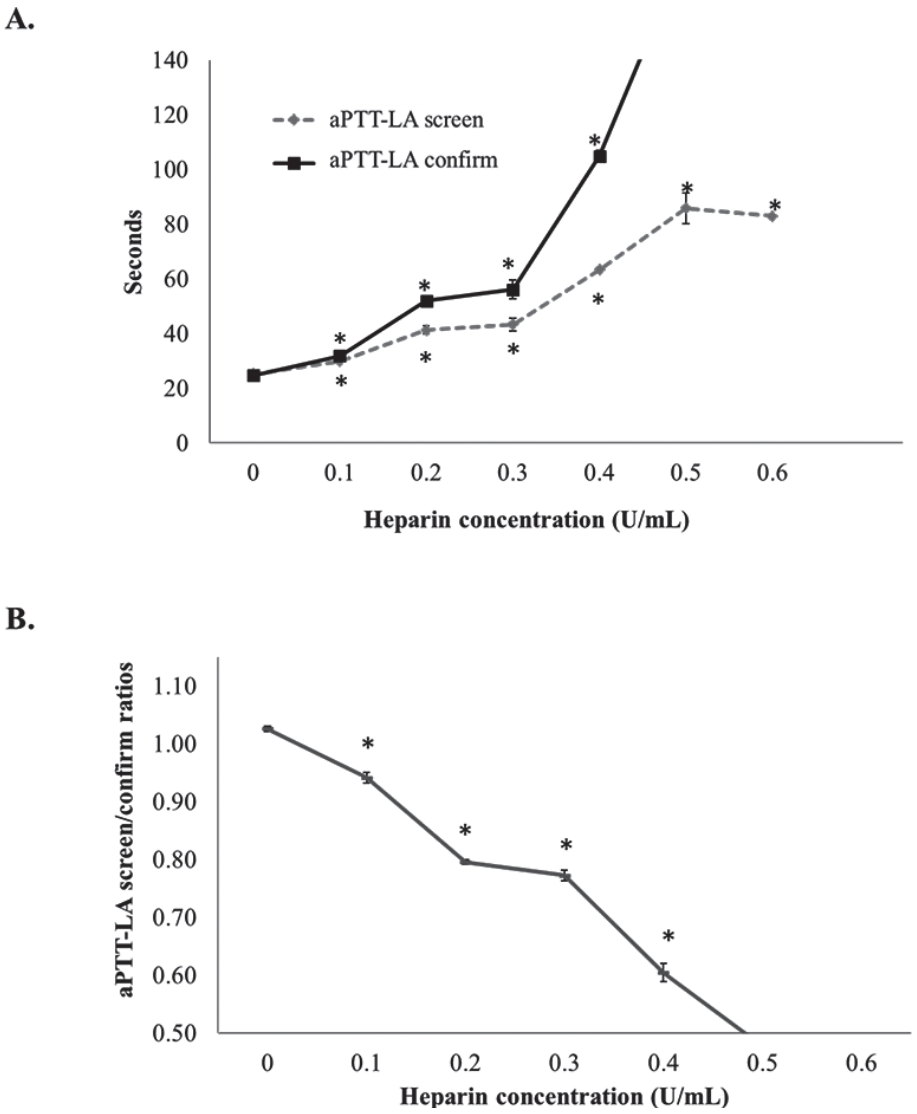

Figure 1 The effects of various concentrations (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 U/mL) of UFH on aPTT-LA screen and aPTT-LA confirm (A), and aPTT-LA screen/confirm ratio (B) * p-value<0.05 when compared with untreated normal plasma controls

The effects of polybrene on aPTT-based LA testing in pooled normal plasma

The aPTT-LA screen and aPTT-LA confirm values were slightly increased after treatment with polybrene in a dose-dependent manner (Figure 2A). The aPTT-LA screen/confirm ratios became slightly lower when higher concentrations of polybrene were added. In the conditions of 0, 15, 20, 25, 30, 35 and 40 μg/mL of polybrene, the aPTT-LA screen/confirm ratios were 1.03±0.005, 0.99±0.004, 0.98±0.004, 0.95±0.005, 0.92±0.002, 0.89±0.003, and 0.85±0.006, respectively (Figure 2B).

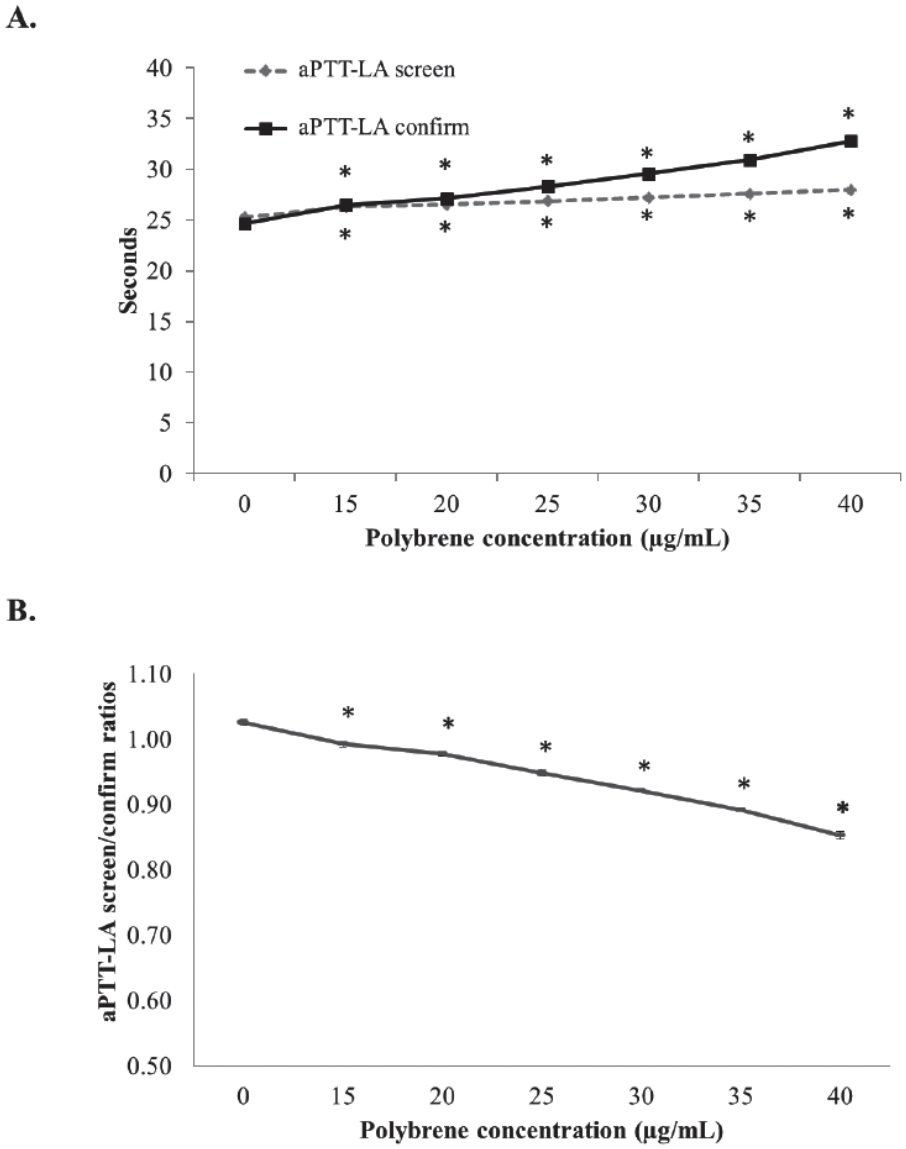

Figure 2 The effects of various concentrations (15, 20, 25, 30, 35, and 40 µg/mL) of polybrene on aPTT-LA screen and aPTT-LA confirm (A), and aPTT-LA screen/confirm ratio (B) * p-value<0.05 when compared with untreated normal plasma controls

The most effective concentrations of polybrene for UFH neutralization

The baseline (mean; 95% CI) aPTT-LA screen/confirm ratio in untreated normal pooled plasmas was 1.03 (1.01-1.04). The results showed that there were three most effective concentrations of polybrene for neutralization of the three concentration ranges of UFH. For neutralization of 0.1 and 0.2 U/mL of UFH, the least mean difference values were shown when treated with 15 µg/mL of polybrene. The aPTT-LA screen/confirm ratios (mean; 95% CI) after UFH neutralization were 1.00 (1.00-1.01) and 0.99 (0.98-1.01), respectively. The least mean difference values for

neutralization of 0.3 and 0.4 U/mL of UFH were found in the mixtures supplemented with 25 µg/mL of polybrene. The aPTT-LA screen/confirm ratios (mean; 95% CI) after UFH neutralization were 1.01 (1.00–1.01) and 1.02 (1.01–1.02), respectively. The most effective concentration of polybrene for neutralization of 0.5 and 0.6 U/mL of UFH was 30 µg/mL. The aPTT-LA screen/confirm ratios (mean; 95%CI) after UFH neutralization were 1.03 (1.02–1.03) and 1.03 (1.01–1.05), respectively (Table 1).

The three most effective concentrations of polybrene for UFH neutralization (15, 25, and 30 µg/mL) were found following the three ranges of aPTT ratios, 1.2–2.0, 2.1–4.0, and >4.0, and the three ranges of anti-factor Xa, 0.3–0.5, 0.6–0.8, and 0.9–1.0 U/mL, respectively. Therefore, this protocol was applied to neutralize UFH in patient plasmas for LA testing in this study (Table 2).

Table 1 The mean differences of activated partial thromboplastin time-lupus anticoagulant (aPTT-LA) screen/confirm ratios after unfractionated heparin (UFH) neutralization with various concentrations of polybrene in pooled normal plasma compared to baseline

UFH concentrations (U/mL)	UFH monitoring		Polybrene concentrations (µg/ml)	aPTT screen/ confirm ratio	
	aPTT ratio Mean (95% CI)	Anti-factor Xa Mean (95% CI) (U/mL)		Mean (95% CI)	Mean difference from baseline Mean (lower, upper)
0	0.96 (0.95–0.96)	0.09 (0.09–0.09)	0	1.03 (1.01–1.04) (Baseline)	–
0.1	1.24 (1.20–1.28)	0.28 (0.28–0.28)	0	0.94 (0.92–0.97)	0.09 (0.05, 0.13)
			15	1.00 (1.00–1.01)	*0.03 (–0.01, 0.07)
			20	0.98 (0.97–1.00)	0.04 (0.00, 0.08)
			25	0.96 (0.95–0.98)	0.06 (0.02, 0.10)
			30	0.92 (0.88–0.96)	0.11 (0.07, 0.15)
			35	0.89 (0.86–0.92)	0.14 (0.10, 0.18)
			40	0.86 (0.81–0.91)	0.17 (0.13, 0.21)
0.2	2.02 (1.83–2.21)	0.46 (0.44–0.48)	0	0.80 (0.79–0.81)	0.23 (0.21, 0.25)
			15	0.99 (0.98–1.01)	*0.03 (0.01, 0.06)
			20	0.98 (0.97–0.99)	0.04 (0.02, 0.07)
			25	0.98 (0.98–0.99)	0.04 (0.02, 0.06)
			30	0.96 (0.93–0.98)	0.06 (0.04, 0.08)
			35	0.93 (0.91–0.94)	0.10 (0.08, 0.12)
			40	0.92 (0.90–0.93)	0.11 (0.09, 0.14)
0.3	2.18 (1.85–2.51)	0.55 (0.53–0.56)	0	0.77 (0.75–0.79)	0.26 (0.22, 0.3)
			15	0.98 (0.98–0.99)	0.04 (0.01, 0.08)
			20	1.00 (0.99–1.01)	0.03 (–0.01, 0.07)
			25	1.01 (1.00–1.01)	*0.02 (–0.02, 0.06)
			30	0.97 (0.95–1.00)	0.05 (0.01, 0.09)
			35	0.95 (0.93–0.96)	0.07 (0.04, 0.11)
			40	0.91 (0.86–0.97)	0.11 (0.08, 0.15)
0.4	4.08 (3.88–4.29)	0.82 (0.77–0.88)	0	0.60 (0.56–0.64)	0.42 (0.39, 0.46)
			15	0.94 (0.92–0.96)	0.08 (0.05, 0.12)
			20	1.00 (0.98–1.02)	0.03 (0.01, 0.06)
			25	1.02 (1.01–1.02)	*0.01 (0.02, 0.05)
			30	1.02 (1.00–1.03)	0.01 (0.03, 0.05)
			35	0.99 (0.97–1.01)	0.04 (0.00, 0.07)
			40	0.97 (0.95–1.01)	0.05 (0.01, 0.08)

Table 1 (continued)

UFH concentrations (U/mL)	UFH monitoring		Polybrene concentrations (μg/ml)	aPTT screen/ confirm ratio	
	aPTT ratio Mean (95%CI)	Anti-factor Xa Mean (95%CI) (U/mL)		Mean (95% CI)	Mean difference from baseline Mean (lower, upper)
0.5	>7	0.94 (0.90-0.98)	0	NC	-
			15	0.87 (0.86-0.89)	0.15 (0.13, 0.17)
			20	0.96 (0.95-0.97)	0.06 (0.04, 0.08)
			25	1.01 (1.00-1.01)	0.02 (0.003, 0.04)
			30	1.03 (1.02-1.03)	*0.00 (-0.02, 0.02)
			35	1.03 (1.02-1.04)	0.00 (-0.02, 0.02)
			40	1.00 (0.98-1.02)	0.03 (0.01, 0.05)
0.6	>7	0.95 (0.90-1.01)	0	NC	-
			15	0.85 (0.84-0.86)	0.18 (0.14, 0.21)
			20	0.92 (0.88-0.96)	0.11 (0.07, 0.14)
			25	1.00 (1.00-1.01)	0.03 (0.01, 0.06)
			30	1.03 (1.01-1.05)	*0.00 (0.03, 0.03)
			35	1.03 (1.01-1.04)	0.00 (0.03, 0.03)
			40	1.01 (0.99-1.03)	0.02 (0.02, 0.05)

*the least mean difference
CI=confidence interval, NC=no coagulation

Table 2 The guide of using polybrene to neutralize unfractionated heparin (UFH) in patient plasmas for lupus anticoagulant (LA) testing

UFH monitoring		Polybrene concentrations for UFH neutralization (μg/ml)
aPTT ratio	Anti-factor Xa (U/mL)	
1.2-2.0	0.3-0.5	15
2.1-4.0	0.6-0.8	25
>4.0	0.9-1.0	30

The effect of polybrene in patient plasmas which received UFH on aPTT-based LA testing

In this study, the most effective concentrations of polybrene were added to assess their effect on neutralizing UFH in the plasma samples of 14 patients. The tests were done for three time points after receiving UFH in each patient. Our results showed that UFH therapy affected the aPTT-LA screen/confirm ratios in four ways. The aPTT-LA screen/confirm ratios at one of the three testing time points in two patients showed the NC code because of over-prolonged aPTT-LA confirm values. These results occurred in the patient samples which had high concentrations of UFH (aPTT ratio >7). When this problem is encountered in general, the use of polybrene at 30 μg/mL can bring the aPTT-LA screen/confirm ratio into testable ranges, 1.11 and 1.08 (Table 3). Three patient samples had a false positive aPTT-LA screen/confirm ratio in at least one of the three testing time points (1.96, 1.78, and 1.37 for patient no. 3, 1.54, 1.59, and 1.71 for patient no. 4 and 1.64 for patient no. 5). The minimal and maximal values of the aPTT ratios of these three patients were 1.83 and 2.39, respectively. For false positives, the use of polybrene at 15 or 25 μg/mL can bring the aPTT-LA screen/confirm ratios into normal range (1.25, 1.20, and 1.22 for patient no. 3, 1.05, 0.92, and 1.0 for patient no.4 and 0.95 for patient no.5) (Table 3). The aPTT-LA screen/confirm ratios at one of the three testing

time points of patients no. 1, 2 and 6 were 0.77, 0.86 and 0.89, respectively, which were lower than the mean−3S.D. (0.91) of the normal range. These false low values were expressed when the plasmas had high concentrations of UFH (aPTT ratios >7, 5.38 and 4.74). For this condition, the use of polybrene at 30 μg/mL can bring the aPTT−LA screen/confirm ratios up to normal range, 0.99, 1.21 and 1.14, respectively (Table 3). The aPTT−LA screen/confirm ratios of the other 8 patients (nos. 7−14) were within the normal ranges, with minimal and maximal aPTT ratios of 1.19 and 3.62 respectively. In these patient samples, polybrene at 15 or 25 μg/mL did not change the LA interpretation from the baseline. In summary, these results indicate that the low to medium concentrations of UFH (aPTT ratio 1.19−3.62) had no effect on LA interpretation in eight of fourteen patients (57.1%) but lead to false positives in three patients (21.4%), while a high concentration of UFH caused no coagulation in two patients (14.3%) and false low results in three patients (21.4%). All of these UFH interference effects can be resolved by neutralizing with the proper concentrations of polybrene.

Table 3 The effect of polybrene for unfractionated heparin (UFH) neutralization in 14 patient plasmas on lupus anticoagulant (LA) testing by activated partial thromboplastin time−lupus anticoagulant (aPTT−LA) assay'

Patient No.	Detection time point	aPTT ratio	aPTT screen/ confirm ratio	
			Without polybrene	With polybrene
1	1	>7	***NC	1.11
	2	>7	*0.77	0.99
	3	1.90	0.99	1.11
2	1	5.38	*0.86	1.21
	2	1.87	1.00	1.16
	3	>7	***NC	1.08
3	1	1.83	**1.96	1.25
	2	1.94	**1.78	1.20
	3	1.98	**1.37	1.22
4	1	1.98	**1.54	1.05
	2	2.20	**1.59	0.92
	3	2.39	**1.71	1.00
5	1	2.38	0.98	0.93
	2	2.26	1.21	1.02
	3	2.13	**1.64	0.95
6	1	2.16	1.14	1.09
	2	4.74	*0.89	1.14
	3	2.19	1.06	0.97
7	1	1.93	1.11	0.99
	2	1.85	1.14	1.04
	3	2.04	1.06	1.04
8	1	1.37	1.20	1.14
	2	1.86	1.21	1.14
	3	2.05	1.28	1.07
9	1	1.72	1.10	1.07
	2	2.24	1.03	0.94
	3	2.62	0.97	0.94

Table 3 (continued)

Patient No.	Detection time point	aPTT ratio	aPTT screen/ confirm ratio	
			Without polybrene	With polybrene
10	1	2.85	1.05	1.09
	2	1.56	1.04	1.03
	3	2.35	1.19	1.11
11	1	2.18	0.93	0.94
	2	1.19	1	0.95
	3	1.75	1.15	1.18
12	1	1.86	1.1	0.93
	2	1.37	1.02	0.96
	3	2.68	1.13	0.97
13	1	2.57	1.06	1.07
	2	3.62	0.98	1.23
	3	2.03	1.03	1.1
14	1	3.44	1.28	1.00
	2	3.5	1.21	0.97
	3	2.84	1.26	0.91

The positive cutoff of aPTT-LA screen/confirm ratio was >1.31.
***NC no coagulation, **False positive, *False low ratio

Discussion

It is well known that heparin contamination can lead to false positives in LA testing.[6,19] Our study reports four patterns of aPTT-LA screen/confirm ratios resulting from the effects of UFH treatment, 1) no coagulation (NC code), 2) false positive, 3) false low ratio, and 4) unchanged interpretation. The different patterns of LA testing results may be affected by many factors such as the limitations of detection by automatic machines, different levels of heparin, varying rates of heparin clearance, between-reagent differences in UFH sensitivity and presence of other factors affecting aPTT such as elevated FVIII.[6] In our study, the different elevation rates between the aPTT-LA screen and aPTT-LA confirm without polybrene in normal pooled plasma samples were likely due, at least in part, to different UFH sensitivities of Actin FSL and Actin FS. A previous study evaluated the sensitivity of Actin FSL and Actin FS to UFH in the plasma of patients with thrombosis. Statistical analysis of the aPTT values in patients undergoing UFH therapy revealed a significant difference between Actin FSL and Actin FS.[20] In addition, false low aPTT-LA screen/confirm ratios in patient plasmas may be due to different sensitivities of FSL and FS to other factors affecting aPTT. A prolonged aPTT suggests deficiency of coagulation factors II, V, VIII, IX, X, XI, XII or fibrinogen. A previous study found that the sensitivities of Actin FSL and Actin FS to assess clotting factor deficiency, including FVIII, FIX, FXI and FXII, were different and influenced by the source of the commercial plasma sample.[21]

In our study, polybrene showed a tendency to slightly prolong the aPTT-LA screen and confirm, similar to a previous study.[22] Polybrene is composed of polycations that neutralize the negatively charged heparin by forming inactive complexes. The anticoagulant effect of polybrene is increased when it is added to citrated plasma. A previous study showed that after a 1:1 ratio of polybrene cationic to

heparin anionic was reached, further polybrene did not form any further complexes or increase anticoagulant activity. The authors suggested that this effect was due to the release of a small amount of heparin from the heparin complex or a change in the configuration of the heparin–polybrene complexes.[23]

A previous study found that 7.9 µg/mL of polybrene could completely neutralize 1.2 units of UFH as determined by a thrombin time assay.[22] Another study found that 25 µg/mL was the optimal final concentration of polybrene for aPTT testing in samples contaminated with UFH.[19] They found that polybrene at 25 µg/mL could neutralize 0.2 to 1.5 U/mL of UFH in normal plasmas with a mean difference of aPTT from –0.33 to 1.87 seconds. To get the most accurate results assessed by the least mean difference from baseline, we used three concentrations of polybrene, 15, 25, and 30 µg/mL, to neutralize three concentration ranges of UFH, 0.1–0.2, 0.3–0.4, and 0.5–0.6 U/mL, respectively. This method generated the most accurate aPTT-LA screen/confirm ratio results with a maximum mean difference of 0.03 seconds when compared to the baseline from untreated normal pooled plasma. Our limitation is unable to study the effects of UFH and polybrene on LA-positive samples, which would have limitations warranting discussion.

Many factors other than UFH can interfere with LA testing. A previous study found that LA positivity by dRVVT and aPTT assays was found in some patients receiving direct oral anticoagulants, 17.0% in patients treated with dabigatran, 50.0% in those treated with rivaroxaban and 41.0% in those treated with apixaban.[24] Another study found that enoxaparin (low molecular weight heparin) and danaparoid (heparinoid) at supratherapeutic ranges lead to false positive LA results. They also found that activated carbon was unable to neutralize the effect of the anticoagulants on LA assays but could cause prolongation of aPTT clotting times.[25]

Conclusion

In conclusion, the best practice for LA testing is doing the tests outside of anticoagulant therapies, but if testing is necessary, the results from our study suggest that the tests can be performed by adding the proper concentration of polybrene.

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Conflict of interest

The authors declare that they have no competing interests.

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