





# Differential Neutralization of Apixaban, Betrixaban, Edoxaban, and Rivaroxaban by Andexanet Alfa as Measured by Whole Blood Thromboelastographic Analysis

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Siddharth Mehrotra<sup>1</sup>, Debra Hoppensteadt, PhD<sup>1,2</sup> , Walter Jeske, PhD<sup>1,2</sup>, Fakiha Siddiqui, BS<sup>2,3</sup>, Omer Iqbal, MD<sup>1</sup>, Alfonso Tafur, MD<sup>4,5</sup>, Bruce Lewis, MD<sup>5</sup>, Mark Jaradeh, BS<sup>1</sup> , Bulent Kantarcioglu, MD<sup>1</sup>, and Jawed Fareed, PhD<sup>1,2</sup> 

## Abstract

**Introduction:** The available oral anti-Xa agents are routinely used for the management of thrombotic disorders. A molecularly modified recombinant coagulation FXa, also known as Andexanet Alfa (AA), that has been developed as an antidote to neutralize the bleeding effects of oral FXa inhibitors, such as Apixaban and Rivaroxaban.

**Materials and Methods:** This study utilized thromboelastography (TEG 5000 Hemostasis System), to investigate the neutralizing effects of AA at different concentrations of oral FXa inhibitors measuring such parameters as R-Time, K-Time, Angle, and Max Amplitude (MA). Apixaban, Betrixaban, Edoxaban, and Rivaroxaban were obtained commercially in powdered form. Each of these drugs was supplemented with freshly drawn whole citrated blood at a concentration of 1 µg/mL. And subsequently mixed with AA at 50 or 100 µg/mL.

**Results:** At a concentration of 1 µg/mL, all FXa inhibitors produced variable anticoagulant effects in the order of Edoxaban > Betrixaban > Rivaroxaban > Apixaban. AA at 100 µg/mL produced a complete neutralization of these inhibitors whereas at 50 µg/mL relatively weaker neutralization as measured by various parameters.

**Conclusion:** These results suggest that regardless of the variable anticoagulant effects exhibited by the FXa Inhibitors, AA at FC = 100 µg/mL fully neutralized these agents as measured by the TEG parameters. AA was shown to be more effective in neutralizing Betrixaban and least effective in Apixaban. The neutralization of various FXa inhibitors was dose and donor-dependent warranting dosage adjustment for optimal outcomes.

## Keywords

anti-Xa agents, anticoagulant, Andexanet Alfa

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<sup>1</sup> Department of Molecular Pharmacology & Neuroscience, Cardiovascular Research Institute, Loyola University Chicago, Health Sciences Division, Maywood, IL, USA

<sup>2</sup> Department of Pathology and Laboratory Medicine, Cardiovascular Research Institute, Loyola University Chicago, Health Sciences Division, Maywood, IL, USA

<sup>3</sup> Program in Health Sciences, UCAM - Universidad Católica San Antonio de Murcia, Murcia, Spain

<sup>4</sup> Department of Vascular Medicine, Northshore Cardiovascular Institute, NorthShore University Health Systems, Skokie, IL, USA

<sup>5</sup> Department of Internal Medicine, Division of Cardiology, Loyola University Medical Center, Maywood, IL, USA

## Corresponding Author:

Jawed Fareed, Department of Molecular Pharmacology & Neuroscience, Loyola University Health System, 2160 S First Avenue Bldg 115, Rm 432 Maywood, IL 60153, USA.

Email: jfareed@luc.edu



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## Introduction

Three oral anti-Xa agents are currently available for clinical use; Apixaban, Edoxaban, and Rivaroxaban, with a fourth agent, Betrixaban, approved but off market. These oral direct factor Xa (FXa) inhibitors are effective anticoagulants and aid in the prevention and treatment of thrombotic events such as thromboembolism. FXa inhibitors bind to FXa and prevent the conversion of prothrombin to thrombin.<sup>1</sup> These FXa inhibitors are also being investigated for efficacy in the treatment of other conditions such as acute coronary syndrome, cancer-associated thrombosis, and peripheral arterial disease.<sup>2,3</sup> Unlike other commonly used anticoagulants, such as heparins and heparin derivatives, FXa inhibitors can be taken orally and do not require any routine or episodic monitoring.<sup>4</sup> However, similar to other anticoagulant options, these anti-Xa agents can cause uncontrolled and life-threatening bleeding.<sup>5</sup> FXa inhibitors such as Rivaroxaban and Apixaban are now widely used in various indications where vitamin K inhibitors such as warfarin were indicated. Despite the widespread use of these agents the bleeding complications are frequently reported and require the use of such antidotes as the four-factor prothrombin concentrates and AA. The efficacy of AA in neutralizing FXa inhibitors is well documented however the dosing regimen widely varies.<sup>6,7</sup> Therefore the circulating levels of these inhibitors may vary during the administration of AA. For this reason, two concentrations of AA were selected in these studies to neutralize the fixed concentration of various FXa inhibitors.

Andexanet Alfa (AA, inactivated-zhzo, coagulation factor Xa) is a recombinant factor Xa protein developed by Portola Pharmaceuticals for use as a universal antidote for the anticoagulant effects of direct or indirect factor Xa inhibitors. Andexanet alfa acts as a decoy protein that binds to FXa inhibitors, neutralizing the anticoagulant effects of FXa inhibitors by preventing the inhibitors from binding to endogenous FXa.<sup>8</sup> In this recombinant protein, the serine active site is replaced by an alanine residue to eliminate catalytic activity and the membrane-binding glutamic acid domain is removed to prevent the assemblage of the prothrombinase complex.<sup>9</sup>

Through accelerated approval, the United States Food and Drug Administration approved the use of AA as an antidote for the reversal of Apixaban and Rivaroxaban anticoagulation in the case of life-threatening or uncontrolled bleeding.<sup>8</sup> Recently, studies have shown AA may also be effective in neutralizing the anticoagulant effects of Betrixaban and Edoxaban, in addition to other anticoagulants, such as enoxaparin and UFH, due to its high-affinity binding to both direct and indirect FXa inhibitors.<sup>10</sup>

While AA is not used or directed as an active procoagulant, its use has been reported to result in thromboembolic events.<sup>5,11</sup> The purpose of this study was to investigate the anticoagulant effects of these FXa Inhibitors and their relative neutralization by AA. Additionally, AA was investigated for its potential of creating a hypercoagulable state.

## Materials and Methods

The active pharmaceutical ingredient (API) forms of various oral anti-Xa agents including Apixaban, Betrixaban, Edoxaban, and

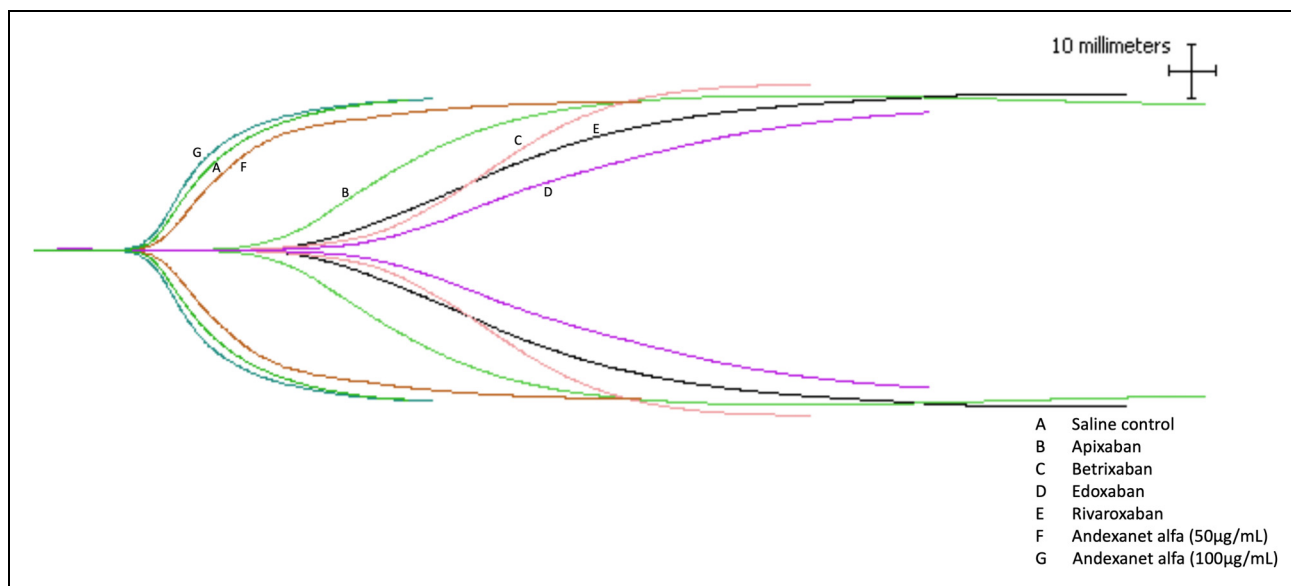
Rivaroxaban were commercially obtained. Working solutions of the oral anti-Xa agents were prepared in saline at 100 µg/mL and stored at four degrees Celsius, working solutions were prepared by diluting to 10 µg/mL at the time of testing. Andexanet Alfa was obtained from the pharmacy service of the Northshore Hospital System. Andexanet Alfa was obtained at a stock concentration of 10 mg/mL in frozen form and thawed immediately prior to the testing to obtain a 1 mg/mL working concentration. In accordance with an Institutional Review Board protocol (9191051098), written informed consent was obtained from all study participants. Native whole blood samples were freshly drawn from healthy donors in individual groups (n = 5-10) into 3.2% citrated tubes. Thromboelastographic (TEG) studies were carried out using TEG 5000 Hemostasis System (Haemonetics Corp, Massachusetts). In each TEG cup for testing, 0.2 M CaCl<sub>2</sub> were added along with FXa inhibitor at 1 µg/mL followed by AA to obtain 50 or 100 µg/mL concentrations. Saline was added to a cup as a control group but also used as a filler for when the FXa Inhibitor or AA were being tested alone. Finally, the citrated human whole blood was added and the test initiated. The final concentrations in the testing system represented the upper limit of anti-Xa agents and levels of AA reflecting circulating concentrations during clinical use. The parameters R-Time, K-Time, Angle, and Max Amplitude (MA) were tabulated along with the thromboelastogram. All results were compiled individually for the saline control, 100 µg/mL AA, and 50 µg/mL AA supplemented systems. All results were tabulated in terms of various parametric measurements in terms of mean ± standard deviation and statistically analyzed using a two-tailed, paired t-test.

## Results

### Comparison of Factor Xa Inhibitors

A composite thromboelastogram of the relative anticoagulant activities of Apixaban, Betrixaban, Edoxaban and Rivaroxaban as compared to the controls represented by saline, and AA at 100 and 50 µg/mL is shown in Figure 1 and showcases the strengths of these anticoagulants relative to one another. AA at 50 µg/mL showed comparable results to the saline control, whereas AA at 100 µg/mL showed a slightly hypercoagulable response.

A direct comparison of the FXa Inhibitors at a final concentration of 1 µg/mL is shown in Table 1. All the FXa agents produced a significant increase in the R-time, K-Time and Angle parameters when compared to the saline control. Edoxaban ( $p < .01$ ) exhibited the strongest anticoagulative effect as measured by the R-time parameter. Next was Betrixaban ( $p < .01$ ) followed by Rivaroxaban ( $p < .01$ ) with Apixaban ( $p < .01$ ) having shown the weakest effect. The order seen in the K Time parameter was different. Edoxaban ( $p < .01$ ) produced the strongest anticoagulative effect, followed by Rivaroxaban ( $p < .01$ ) and then Betrixaban ( $p < .01$ ), with Apixaban ( $p < .01$ ) having produced the weakest effect. The ranked order for strength of effect for the Angle parameter is Edoxaban ( $p < .01$ ), Betrixaban ( $p < .01$ ), Rivaroxaban ( $p < .01$ ), and Apixaban ( $p < .01$ ), respectively. This ranked order for Angle follows the pattern seen in the



**Figure 1.** Thromboelastographic profile of Various anti-Xa agents as compared to controls.

**Table 1.** Comparison of Factor Xa Inhibitors n = 10.

Drug	R-time (minutes)	K-time (minutes)	Angle (degrees)	MA (mm)
Saline Control	12.37 ± 3.74	4.67 ± 2.33	41.46 ± 14.69	54.78 ± 10.22
Apixaban FC = 1 µg/mL	23.91 ± 6.02	9.94 ± 3.48	24.76 ± 10.55	52.18 ± 11.75
Betrixaban FC = 1 µg/mL	30.22 ± 8.65	13.71 ± 4.65	18.67 ± 8.57	50.11 ± 10.38
Edoxaban FC = 1 µg/mL	33.98 ± 13.16	16.5 ± 6.55	15.71 ± 9.89	48.39 ± 11.48
Rivaroxaban FC = 1 µg/mL	29.08 ± 10.65	14.48 ± 8.45	20.41 ± 12.04	48.18 ± 11.36

R parameter. The MA values for all four agents were comparable and ranged from 48.2 to 52.2 mm. Of the FXa Inhibitors, Edoxaban showed the strongest anticoagulative effects while Apixaban showed the weakest effect in all parameters.

**Andexanet Alfa as a Potential Hyper Coagulant**

Shown in Figure 2 are the effects AA had when added to blood alone compared to the saline. No significant differences in any parameter were noted between the saline control and the AA supplemented systems. No consistent trend suggesting AA causes hypercoagulation at either concentration was observed.

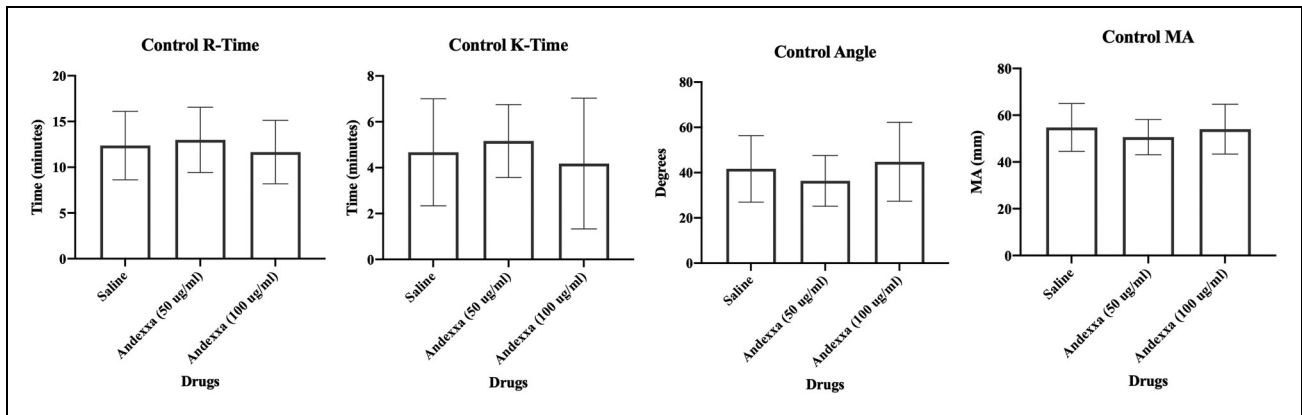
**Andexanet Alfa at 50 µg/mL**

Figure 3 shows a comparison of AA at 50 µg/mL and the neutralizing effects on the various anti-Xa agents at 1 µg/mL. Each oral anti-Xa agent produced an anticoagulant effect and when supplemented with AA there was a varying decrease in the measured parameters. As shown in Figure 3, Apixaban and Edoxaban were not as effectively neutralized by 50 µg/mL AA when compared to Betrixaban and Rivaroxaban in terms of maximum amplitude. Each anti-Xa agent produced a varying anticoagulant effect, and the addition of AA produced a variable neutralizing effect.

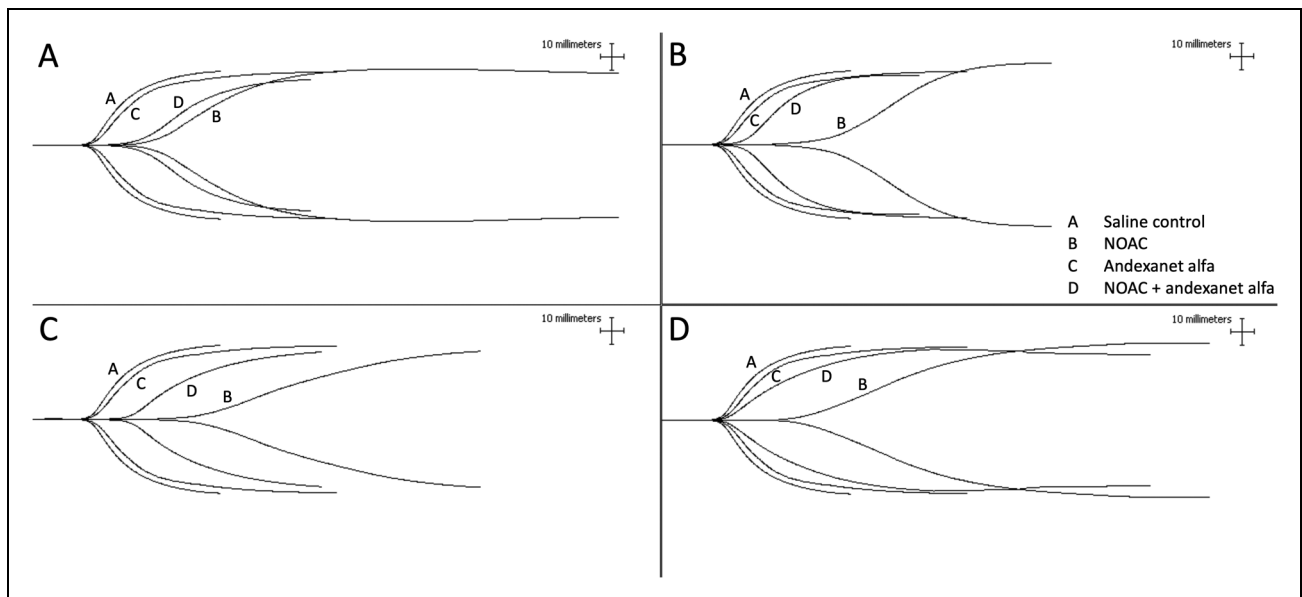
Table 2 compares AA-supplemented systems with the various anti-Xa agent as compared to the saline control. With respect to R-time, in the presence of AA, the Rivaroxaban and Betrixaban systems, respectively, were most comparable to the control value. When supplemented by AA, Edoxaban and Apixaban, respectively, then followed and had very similar R-times to one another (20.44 min). In regard to the K-Time parameter, when supplemented with AA, Betrixaban was the most comparable to the saline control followed by Rivaroxaban, Edoxaban, and then Apixaban, respectively. In regard to Angle parameter, the Betrixaban-AA system was the closest to the saline control value followed by the Rivaroxaban-AA system. The Apixaban- and Edoxaban-AA systems then followed but had very similar values to one another (22.46 degrees). This trend was also seen in R-time. In the MA parameter, the four anti-Xa agent and AA supplemented systems were relatively consistent with one another. Compared to the saline control, the Betrixaban-AA system was the closest followed by Rivaroxaban-AA, Apixaban-AA, and Edoxaban-AA systems, respectively.

**Andexanet Alfa at 100 µg/mL**

Figure 4 compares AA at a concentration of 100 µg/mL and its neutralization of the four factor Xa inhibitors. The anti-Xa



**Figure 2.** Aa alone compared to saline control.



**Figure 3.** Thromboelastographic representation of AA neutralization at 50  $\mu\text{g/mL}$ .

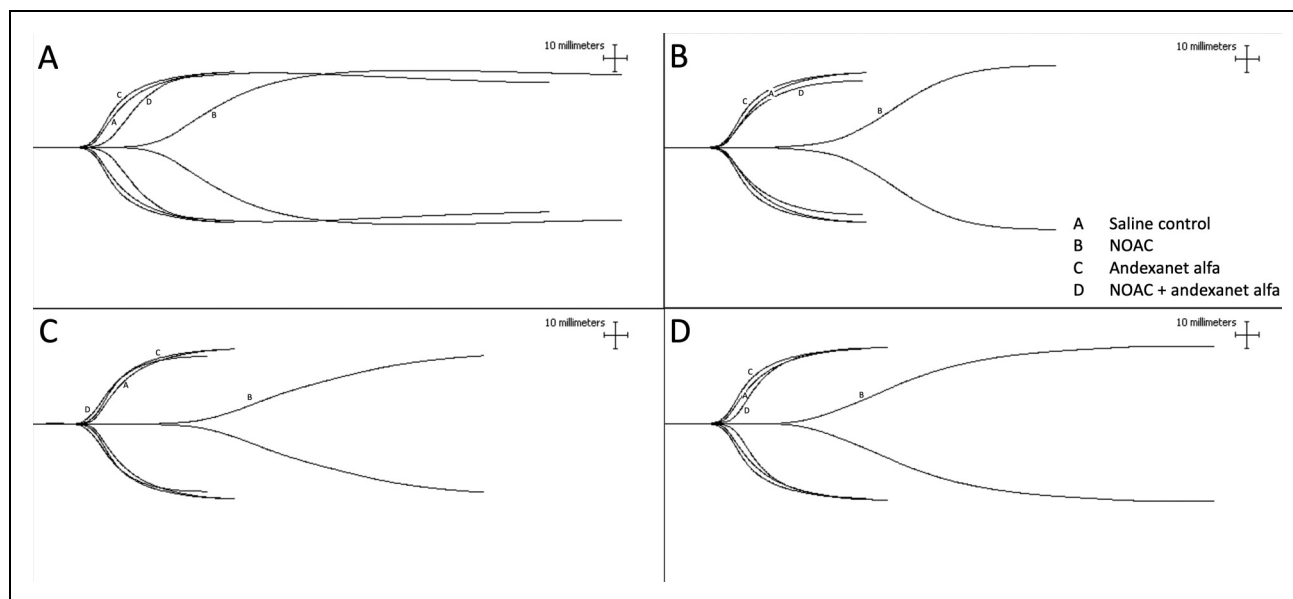
**Table 2.** Effectiveness and Differential AA Neutralization at 50  $\mu\text{g/mL}$ .

Drug (AA FC = 50 I $\mu\text{g/mL}$ )	R-time (minutes)	K-time (minutes)	Angle (degrees)	MA (mm)
Saline Control	12.37 $\pm$ 3.74	4.67 $\pm$ 2.33	41.46 $\pm$ 14.69	54.78 $\pm$ 10.22
Apixaban (FC = 1 $\mu\text{g/mL}$ ) + AA	20.44 $\pm$ 3.59	9.67 $\pm$ 3.66	22.46 $\pm$ 8.16	47.4 $\pm$ 8.14
Betrixaban (FC = 1 $\mu\text{g/mL}$ ) + AA	16.32 $\pm$ 3.66	6.48 $\pm$ 2.39	33.82 $\pm$ 12.28	48.34 $\pm$ 7.57
Edoxaban (FC = 1 $\mu\text{g/mL}$ ) + AA	20.44 $\pm$ 2.68	8.02 $\pm$ 2.15	22.46 $\pm$ 8.47	44.84 $\pm$ 8.46
Rivaroxaban (FC = 1 $\mu\text{g/mL}$ ) + AA	13.94 $\pm$ 5.23	7.88 $\pm$ 5.13	28.66 $\pm$ 13.57	47.76 $\pm$ 9.49

agents produced differing anticoagulant effects, however, when supplemented with AA, the results were comparable to that of the saline control. The collection of thromboelastographs suggests Apixaban neutralization by AA was the least comparable to the saline control relative to the other anti-Xa agents.

Table 3 shows the AA neutralization of these anti-Xa agents in a tabulated form. For R and K-time in this representation, the Apixaban and AA system is the least comparable to the saline control. Both the Betrixaban and Edoxaban AA-supplemented

systems were slightly faster compared to control. In the K-time parameter, the Betrixaban-AA, Edoxaban-AA, and Rivaroxaban-AA systems were very similar to the saline control value. In the Angle parameter, the Edoxaban and Rivaroxaban AA supplemented systems were slightly faster when compared to the control. The Apixaban-AA and Betrixaban-AA systems were slightly slower compared to the control value. In MA, the Apixaban-AA system was most similar to control. The Betrixaban-AA, Edoxaban-AA, and



**Figure 4.** Thromboelastographic representation of AA neutralization at 100 µg/mL.

**Table 3.** Effectiveness and Differential AA Neutralization at 100 µg/mL.

Drug (AA FC = 100   µg/mL)	R-time (minutes)	K-time (minutes)	Angle (degrees)	MA (mm)
Saline Control	12.37 ± 3.74	4.67 ± 2.33	41.46 ± 14.69	54.78 ± 10.22
Apixaban (FC = 1 µg/mL) + AA	14.44 ± 4.45	5.76 ± 4.15	37.88 ± 21.53	54.48 ± 10.32
Betrixaban (FC = 1 µg/mL) + AA	11.98 ± 5.05	4.72 ± 2.73	37.34 ± 17.69	51.64 ± 10.29
Edoxaban (FC = 1 µg/mL) + AA	10.68 ± 4.54	4.54 ± 2.89	42.46 ± 21.43	51.52 ± 11.75
Rivaroxaban (FC = 1 µg/mL) + AA	13.52 ± 3.8	4.5 ± 2.35	42.56 ± 15.05	51 ± 10.8

Rivaroxaban-AA systems were slightly less when compared to the control. MA values of Betrixaban-AA, Edoxaban-AA, and Rivaroxaban-AA systems were similar. Overall, the results in all parameters were similar and comparable to the control value. Considering the variation by individual donors, the differences noted and mentioned are not significant.

### Comparison of AA Neutralization at 50 and 100 µg/mL

Figure 5 compares the effectiveness of AA in neutralizing the anti-Xa agents at the two different concentrations of 50 and 100 µg/mL. Comparing the anti-Xa agents (labeled as Drug Control), Edoxaban is seen to have the strongest anticoagulant effect as measured in all the parameters. This is followed by Betrixaban and Rivaroxaban, which were similar in all parameters. Apixaban in this study was noted to have the weakest anticoagulant effect of the four anti-Xa agents tested.

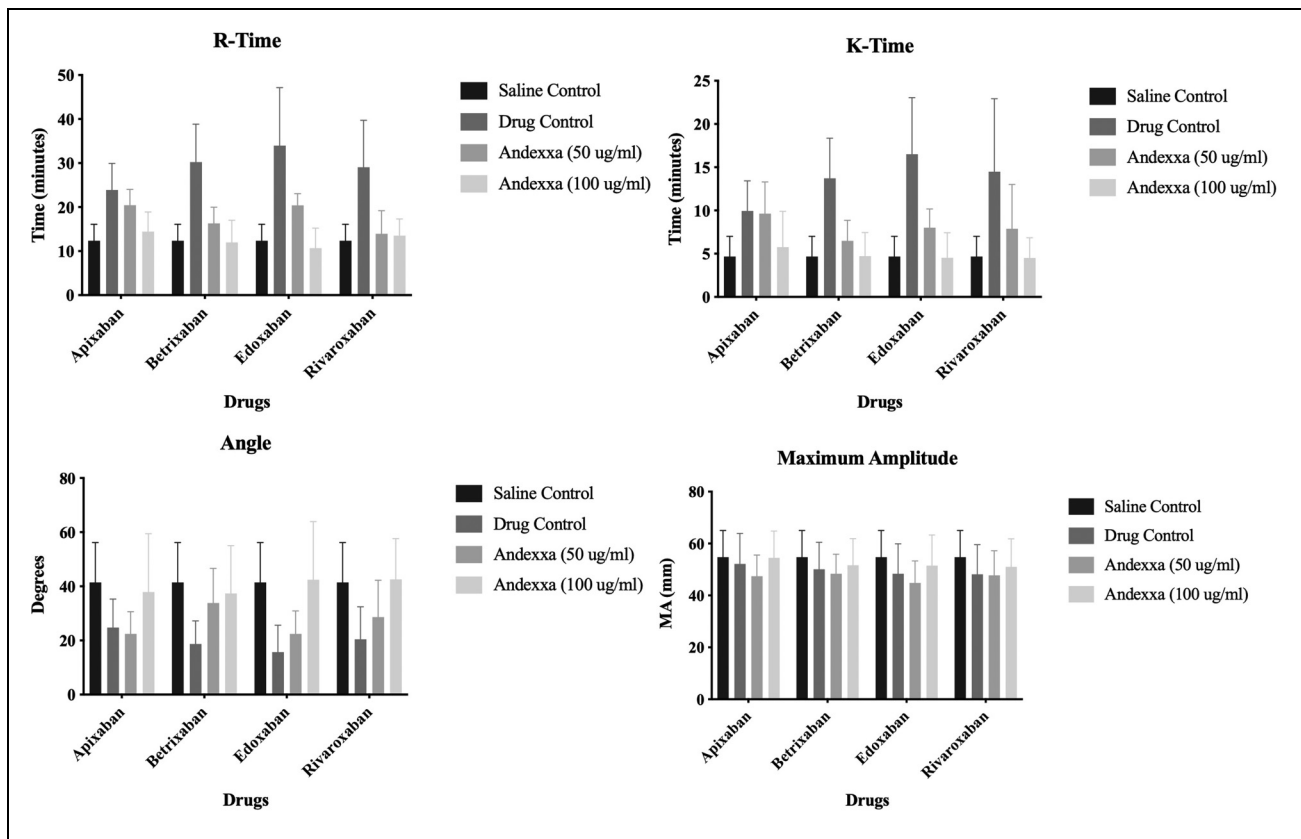
In R-Time and K-Time, AA neutralization of Apixaban was the least effective at either concentration. In R-time, a stronger and more consistent neutralizing effect by AA was observed at a concentration of 100 µg/mL when compared to the 50 µg/mL results. A similar trend is seen in the K-time, Angle, and MA parameters. In the MA parameter, we see that, when AA was supplemented to the anti-Xa agent at 50 µg/mL, there are

slight additional anticoagulant effects seen when AA is added as the MA value is lower than that of the anti-Xa agent alone.

### Discussion

AA is an FDA-approved antidote for the neutralization of the anti-Xa effects of available direct oral anticoagulants, such as Apixaban and Rivaroxaban. This study was carried out to compare the relative efficacy of AA in neutralizing not only Apixaban and Rivaroxaban, but also the other orally bioavailable anti-Xa agents, such as Betrixaban and Edoxaban. Unlike the other studies which have used Factor-Xa inhibition as an indicator for the neutralization effects, this study utilized a whole blood thromboelastographic assay.<sup>2,3</sup> The thromboelastographic assay is relatively sensitive and can be used for the monitoring of clinical levels of Apixaban and Rivaroxaban. Two concentrations of AA, 50 and 100 µg/mL, representing clinically relevant serum levels as determined by pharmacokinetic studies of its recommended dosing regimens were selected for these studies.<sup>1</sup>

A fixed concentration of various FXa inhibitors at 1 µg/mL was chosen to reflect the supramaximal dosage of these inhibitors which may result in hemorrhagic complications. The usual circulating therapeutic levels of these agents for various



**Figure 5.** Effect of AA at 50 and 100 µg/mL on oral anti-Xa agents.

therapeutic indications ranges from 100 to 500 ng/mL. Therapeutic and subtherapeutic levels of FXa inhibitors do not require neutralization in normal circumstances. In these studies, the fixed concentrations of FXa inhibitors at 1 µg/mL were selected to represent supratherapeutic concentrations which would require neutralization. This supratherapeutic concentration of FXa inhibitors was selected to test the efficacy of AA in neutralizing these agents in the whole blood system. The two concentrations selected for AA at 50 and 100 µg/mL represent the projected concentrations which can be reached during the use of this agent and provided two points where the effect of this agent can be differentiated. Due to the limitation of blood availability this study was limited to include fixed concentrations of FXa inhibitors and their respective neutralization at two concentration of AA.

The thromboelastographic profiles of various anti-Xa agents were comparable and each of these agents produced a measurable anticoagulant effect. Edoxaban was found to have the strongest anticoagulant effect while Apixaban had the weakest anticoagulant effect. Betrixaban and Rivaroxaban had similar effects. Various TEG parameters, such as R-time, K-time, Angle, and MA showed proportionate changes in comparison to Saline. R-time measures the time it takes for the sample to initiate the formation of a clot. K-time measures the time it takes from the initial formation of the clot to reach an amplitude of 20 mm. Angle measures the rate of clot formation and MA measures the strength of clot formation. Edoxaban

produced the most pronounced increase in R-time. Whereas Apixaban was relatively weaker. Similar results were found when the K-times were compared. The angle values showed the differences among these agents in the reverse order. The MAs were in the range of 48 to 54 mm. In these studies, wide individual variations from individual donors were noted. This may likely be due to the composition of blood and its components. In addition, such variations may be due to such variables as the time of blood draw and duration of the testing, endogenous components of blood and their contributions to the testing procedure and binding of the drugs to plasma proteins. Since this is a whole blood method, the reproducibility of the technique may also have been contributory to the observed variability in the test results. Such variations can be further studied in carrying out these studies in larger populations.

AA at either concentration did not have any significant effect on the thromboelastographic profile as compared to the saline control. AA at 100 µg/mL had negligibly quicker R and K times with a higher Angle whereas AA at 50 µg/mL had negligibly slower R and K times with a lower Angle.

At a final concentration of 50 µg/mL, AA produced varying levels of neutralization of the TEG profile of blood supplemented at 1 µg/mL of each of these agents. AA at 50 µg/mL was effective at neutralizing these agents in the order Rivaroxaban, Betrixaban, Edoxaban, and Apixaban. For R-time, the Rivaroxaban-AA system was the most comparable

to the saline control with less than a two-minute difference. This indicates that this lower concentration of AA can potentially be used to effectively reverse the anticoagulant effects of Rivaroxaban. In the K-time parameter, the Betrixaban-AA system is the most neutralized while Apixaban is the least neutralized. This same trend is seen in the Angle parameter. For the MA parameter, Apixaban, Betrixaban and Rivaroxaban had comparable neutralization while Edoxaban had the weakest clot formation.

Comparatively, AA at 100 µg/mL had complete neutralization of each of the agents. At this concentration, there is a stronger and less variable reversal effect. In the agents Betrixaban and Edoxaban, R-time measurements suggested faster coagulation when compared to the saline control. This indicates “over-neutralization,” or a relative hypercoagulable state, and suggests it is possible to potentially administer too much AA for the purpose of neutralization, potentially leading to adverse effects. For the R-time, all agents were reversed to be within two minutes of the saline control value. This trend is similar for K-time in that all the agents are brought within one minute of the saline control value. For the Angle parameter, it can be noted that the rates of clot formation are relatively similar, with Edoxaban and Rivaroxaban being slightly faster than the saline control and Apixaban and Betrixaban being slower. The MAs for the systems at this concentration of AA are also all similar, with Apixaban being the most comparable to the saline control.

In comparing the R-times and the differences between neutralizing effects of AA at 50 and 100 µg/mL, there is a significant difference in efficacy. AA at 50 µg/mL partially neutralizes the anticoagulant effects of Apixaban, Betrixaban, and Edoxaban, but in Rivaroxaban shows a full neutralization effect with respect to the R-time parameter. AA at 100 µg/mL fully neutralizes the anticoagulant effects of all the anti-Xa agents, bringing the R-times to near the Saline control. In K-Time, there is a partial neutralization effect seen for all the anti-Xa agents when AA is added at 50 µg/mL and full neutralization effects when AA is added at 100 µg/mL. In the Angle parameter, there is no neutralization seen when AA is added at 50 µg/mL to Apixaban, but a slightly stronger anticoagulant effect is seen. In the other agents, when AA at 50 µg/mL is added, there is slight neutralization noted in Edoxaban and partial neutralization in Betrixaban and Rivaroxaban. AA at 100 µg/mL in the Angle parameter has a complete reversal of the anti-Xa agents. In the MA parameter for AA at 50 µg/mL, there are slightly more anticoagulant effects seen when added to the anti-Xa agent. The MA drops in Apixaban, Betrixaban, and Edoxaban with no change in Rivaroxaban. When AA is added at 100 µg/mL, there is only complete neutralization seen in Apixaban with only partial neutralization in Betrixaban, Edoxaban, and Rivaroxaban. However, with all the MA values ranging from 48 to 54 mm, overall there is not a major difference in the clotting profile with regard to clot strength.

Apixaban had the weakest anticoagulant effect but was also the least neutralized by AA at both concentrations. Looking at

the R-time parameter, it can be noted that when AA is added at 100 µg/mL, the time is 14.44 min while the saline control is at 12.37 min while the other agents are less than the saline control or within a single minute of the saline control value. For the K-time parameter, when AA is added at 100 µg/mL to Apixaban, the resulting neutralization remains a minute slower while all the other agents are comparable with the saline control. AA at 50 µg/mL shows a similar trend with Apixaban. Regarding R-time and K-time, the Apixaban-AA system lags in comparison to Betrixaban and Rivaroxaban. This leads to further questions as to why AA at both concentrations neutralizes Apixaban the least when it has the smallest anticoagulant effect of the agents.

The assay-based differentiation in the neutralization of heparin-based drugs has been previously reported.<sup>12</sup> Other groups have also found that the Factor Xa inhibitory profile of Apixaban, Betrixaban, Edoxaban, and Rivaroxaban was not reflective of their biological spectrum.<sup>3</sup> The results of these studies demonstrate different orders of anti-Xa effects in contrast to our studies. Therefore, assay-based differences should be considered while the neutralization of these drugs is investigated. The TEG represents a whole blood assay where the blood, plasma, and its components contribute to the biological effect of these drugs and is likely to be more representative of the endogenous effects observed in clinical conditions.

The reversal of direct FXa inhibitors by Andexanet has also been reported using other assays such as the ACT, Clot-based PT and aPTT, anti-Xa, anti-IIa, and the inhibition of thrombin generation. In these assays, varying degrees of neutralization were noted for each of these direct Xa agents. The relative neutralization of the anti-Xa effects is also agent-specific.<sup>2</sup> Some of the assays have also demonstrated the procoagulant effect of Andexanet. For endogenous pharmacological effects and potential bleeding, the whole blood assay, such as ACT and TEG, may be more useful.

Several recent studies have compared prothrombin complex concentrates and Andexanet for the reversal of Apixaban and Rivaroxaban in clinical populations and experimental studies.<sup>13–15</sup> Studies have also suggested Andexanet Alfa can partially neutralize heparin-related drugs through assays such as ACT.<sup>16</sup> Heparin resistance has been reported in patients where the effect of direct oral anti-Xa agents is fully neutralized.<sup>17</sup> It is quite clear that in some of the assays, even at concentrations between 50–100 µg/mL, measurable biological activity persists. The differential neutralization of these agents in different assays is suggestive of target-specific inhibitory effects of not only these agents, but also when Andexanet is administered. Animal models of bleeding and thrombosis may be needed to elucidate these differences and further evaluate the relevance of the true biological effects. The current studies reported show that assays relying on whole blood, such as TEG, may be more relevant to the physiologic conditions in therapeutic, subtherapeutic, and supratherapeutic states.<sup>12</sup> While plasma-based assays may be useful for standardization, they have been proven to have limitations with regard to

predicting the biological actions of these drugs.<sup>12</sup> Advanced versions of TEG-like techniques, such as rotational thromboelastometry and other modified TEG techniques, may be viable but currently require further clinical validation.

This study has several limitations such as a limited number of subjects recruited, use of fixed concentration of the anti-Xa agents, and no parallel comparison with ACT and other plasma-based assays. Thus, wide intraindividual variations were noted in the TEG results. Moreover, these studies were carried out in ex-vivo settings and were primarily designed to demonstrate the efficacy of AA in altering the TEG parameters to reflect the neutralization effects of this agent. Furthermore, the dosing regimen of these agents widely differ and may reach different concentrations to produce therapeutic and supratherapeutic effects. There are large differences in the dosages of each agent and the frequency, moreover, the pharmacokinetics of each agent follows different patterns. Additionally, the relevance of the in-vitro neutralization to the in-vivo is not clear, however, the use of whole blood is more relevant to the in-vivo settings as compared to plasma or other artificial systems. This study did not take into account the complete blood profile and the effect of cellular components such as red cells. Nevertheless, the utility of TEG in potential future studies in clinical settings can validate some of these observations. Despite these limitations, our study clearly demonstrates that TEG is a reliable method to compare the anticoagulant effects of the direct oral anti-Xa agents and truly reflects their neutralization by Andexanet Alfa. This study further demonstrates that Andexanet produced a concentration-dependent neutralization of the biological effects of these drugs as measured by calculating and monitoring the TEG profile and parameters.

## Conclusion

Direct oral anti-Xa agents, such as Rivaroxaban and Apixaban, are widely used in the management of venous thromboembolism and other related disorders. Bleeding complications with their use have been observed in certain patient populations, especially in the elderly and those with lower body mass. AA represents the very first specific antidote to neutralize the anti-Xa effects of these agents. This neutralization results in the decrease or loss of the pharmacological effects in addition to the potential bleeding associated with the use of these agents. In this study, a whole blood thromboelastographic approach has revealed differential anticoagulant effects of these agents which are partially neutralized at 50 µg/mL concentrations of Andexanet Alfa and complete neutralization at 100 µg/mL. As the TEG considers whole plasmatc and cellular factors contributing to the pharmacological effects of anti-Xa agents, this study provides an approach to monitoring the circulating levels of direct oral anti-Xa agents. These studies may allow for more appropriate dose adjustments and monitoring in clinical practice.

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## Consent

Written informed consent was obtained from the patient(s) for their anonymized information to be published in this article.


## Declaration of Conflicting Interests


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## ORCID iDs

Debra Hoppensteadt  <https://orcid.org/0000-0001-9342-4213>

Mark Jaradeh  <https://orcid.org/0000-0001-8916-7533>

Jawed Fareed  <https://orcid.org/0000-0003-3465-2499>

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