Introduction
Thromboelastographic (TEG) analysis represents a global approach to monitoring the clottability of native whole blood and is sensitive to the cellular and plasma components of blood which in turn affect the rate of formation, structure, and breakdown of a thrombus. This method can be used for the evaluation of the point of care hemostatic status of whole blood. rTM (recombinant Thrombomodulin) is currently being developed for various clinical indications as it exerts multiple actions on blood and its components to reduce thrombogenesis without increasing bleeding risk. This is in contrast to heparins which also reduce thrombogenesis but are accompanied by some bleeding risk. FEIBA (Factor VII Inhibitor Bypass Activity) contains non activated Factors II, IX and X and activated factor VII. It has been approved by the FDA for routine prophylaxis in Hemophilia A and B patients with inhibitors and to prevent or reduce the frequency of bleeding episodes. The purpose of this study was to compare the anticoagulant effects and the associated bleeding risk of rTM and Unfractionated Heparin (UH) at various levels and their neutralization by FEIBA.

Methods
Citrated Whole Blood samples were supplemented with rTM or UH in a concentration range of 0-10 µg/ml. (n=10). TEG analysis was performed on a TEG 5000 system in which the clotting process was initiated by reactivation of the whole blood. The parameters R time, K Time, MA and Angle were measured. The relative neutralization profiles of UH and rTM by FEIBA at 1 and 0.1 U/ml were also investigated. All results are reported in terms of mean ± standard deviation.

Results
In comparison to UH, rTM exhibited much weaker anticoagulant effects which were evident in all parametric evaluations. At 1 µg/ml rTM did not produce any anticoagulant effects as evident by TEG analysis. At levels greater than 2.5 µg/ml, rTM produced a concentration dependent anticoagulant effect which altered the TEG profile of the whole blood. In contrast, UH produced relatively stronger anticoagulant effects and at a concentration greater than 2.5 µg/ml, totally inhibited the clot formation in the TEG analysis. At higher concentrations, UH produced strong anticoagulation of whole blood whereas rTM at a concentration of 10 µg/ml produced weaker effects. FEIBA at a concentration of 1 U/ml completely neutralized the anticoagulant effect of rTM at 10 µg/ml and UH at 0.5 µg/ml. Furthermore, FEIBA at a concentration of 0.1 U/ml neutralized the anticoagulant effects of rTM completely at a concentration of 10 µg/ml. At this level, it only partially neutralized the anticoagulation effects of heparin at 0.5 µg/ml.

Conclusions
These studies suggest that rTM is a relatively weaker anticoagulant in comparison to UH. Furthermore, in the given indications, the expected concentration of rTM is in the range of 0.5-1.5 µg/ml. At these concentrations, this agent is not expected to produce any anticoagulant effects. In contrast, UH at therapeutic concentrations of 1-2 µg/ml produces strong anticoagulant effect. While these TEG studies indicate that rTM is an antithrombotic agent mediating its effects via multiple mechanisms, heparins primarily produce anticoagulant effects. Moreover, the superantithrombotic levels of thrombomodulin and heparin can be neutralized by FEIBA.

![Figure 1: Anticoagulant effects of rTM and Heparin both at a final concentration of 1 µg/ml as measured by TEG.](image)

**Table A** represents the comparison of anticoagulation effects of rTM and UH both at a concentration of 1 µg/ml.

**Table B** represents the neutralization of the anticoagulation effects of rTM(10 µg/ml) and heparin(0.5ug/ml) by FEIBA(1 U/ml).

* Both tables represent coagulation parameter calculated using TEG.

![Graphs A,B,C and D](image)

**Graphs A, B, C and D** are bar charts representing different parameters of coagulation as measured by TEG showing comparison of anticoagulant effects of rTM and heparin at 1µg/ml. A - R time (time taken for coagulation to start); B - K time (time taken for coagulation to take place); C – Angle; D - MA (strength of the clot)

**Graphs E, F, G and H** are bar charts representing different parameters of coagulation as measured by TEG showing the neutralization of the anticoagulation effects of rTM(10µg/ml) and heparin(0.5µg/ml) by FEIBA(0.1 U/ml).

**E** - R time (time taken for coagulation to start); **F** - K time (time taken for coagulation to take place); **G** – Angle; **H** – MA (strength of the clot)

**Clinical Implications**
FEIBA at a concentration of 0.1U/ml is capable of neutralizing the anticoagulant effects of heparin and thrombomodulin effectively. Thus lower dosages of FEIBA can be used, to neutralize the potential anticoagulant effects of rTM. Since FEIBA is an approved hemostatic agent it is readily available and can be used for the rare bleeding complications which may be observed with very high dosages of rTM.