

# IN VITRO MODELING OF THE INFLUENCE OF FVIII ACTIVITY AND HEPARIN INDUCED PROLONGATION OF APTT

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## ABSTRACT

Anticoagulant therapy is most commonly assessed by measuring the effect of the drug on global clotting assay, such as APTT. It is known that response of the APTT to heparin may be decreased in patients with high levels of factor VIII. In this work, we have attempted to determine *in vitro* conditions of experiment for obtaining relationship between different concentrations of heparin and values of APTT, and to investigate influence of factor VIII on correlation between concentrations of heparin and APTT. Measurement of the effect of heparin, added *in vitro* in normal coagulation control plasma (NCCP) showed that heparin in concentrations from 0,1 to 1,0 IU/mL prolonged APTT from 0,73 s to 99,26 s. Linearity of the relation of natural logarithm of APTT and concentration of added heparin in plasma for concentrations from 0,5 to 1,0 IU/mL ( $r = 0,995$ ), and other characteristics of the validated method (RSD = 1,17%), made possible investigation of the influence of factor VIII addition in the solution. The addition of the Factor VIII concentrate, markedly influenced these APTT results. Increased factor VIII activity shortened the APTT, having more pronounced effect in the presence of the large amounts of heparin. Increased factor VIII was associated with downward shift in the concentration - logAPTT response curve ( $y = 24,644 x + 30,17$  vs.  $y = 10,864 x + 27,256$ ). This finding suggests the possibility for modeling of *ex vivo* establishment of correlation between plasma activity of FVIII and needed doses of heparin for appropriate management of heparin therapy.

**KEY WORDS:** heparin, APTT, factor VIII

## INTRODUCTION

Literature data about the relation of FVIII level in plasma with thromboembolic disease already exist (1,2). Implication of F VIII level in reoccurrence of the disease is verified and the level was set up to 234 IU/dL (3). Despite the unknown underlying mechanism which is in origin of of F VIII increased level in thromboembolic disease (2), its influence on shortening of the initial phase of the coagulation (1), and speed of thrombin generation (4), have been confirmed. In attempt to establish mathematical correlation for modeling of relationship of increased FVIII level and its effect on heparine induced prolongation of activated partial tromboplastine time one in vitro method was validated.

## MATERIAL AND METHOD

Activated Partial Thromboplastine Time (APTT) was determined by clotting method (Dialab, DiaChrom C4 combi). This investigation was performed with commercial plasma (Normal Coagulation Control Plasma – NCCP). Activity of FVIII in NCCP was determined by one-stage assay, routinely employed for this purpose in clinical practice, and measured 97,8 %, which is equivalent to 0,98 IU/mL. This plasma was after spiked with theoretical activity of 1,0; 2,0; 3,0; 4,0 and 5,0 IU/mL of commercial preparation of FVIII (Haemate<sup>®</sup>P), and in the results figures as addition of before specified activities. Basal value of NCCP – APTT was  $33,55 \pm 0,39$  s, with 1,17% of variation of eight determinations, that was found as acceptable reproducibility located within the limits of the specification sheet (32,0-37,0 s). It was decided that all experiment should be performed within 8 hours of claimed stability of NCCP. Volumes, handling of samples and duration and temperatures of incubation, were designed in accordance with general principle of the method. USP heparin sodium reference preparation (labeled activity of 167,22 IU) was used in the investigation. By appropriate dilution one stock solution of 10 IU/mL heparin sodium was prepared, which was utilized for further standard dilutions of 0,1 IU/mL, 0,2 IU/mL, 0,4 IU/mL, 0,5 IU/mL, 0,6 IU/mL, 0,8 IU/mL and 1,0 IU/mL. The values of APTT for each concentration level was calculated as a mean of eight determinations. On the basis of calibration of the method it was decided that the concentration of 0,5 IU/mL, located between 1,5 and 2,5 prolongation of the basal value of the APTT will be used for the investigation. APTT values for the concentrations of heparin so-

dium of 0,1;0,2; 0,4; 0,5; 0,6; 0,8 and 1,0 IU/mL were 34,27 s, 37,65 s, 50,10 s, 58,55 s, 76,05 s, 99,71 s, and 132, 81 s, respectively. From the calibration curve the value of 0,5 IU/mL was chosen as needed for the two fold prolongation of the basal APTT (Figure 1).

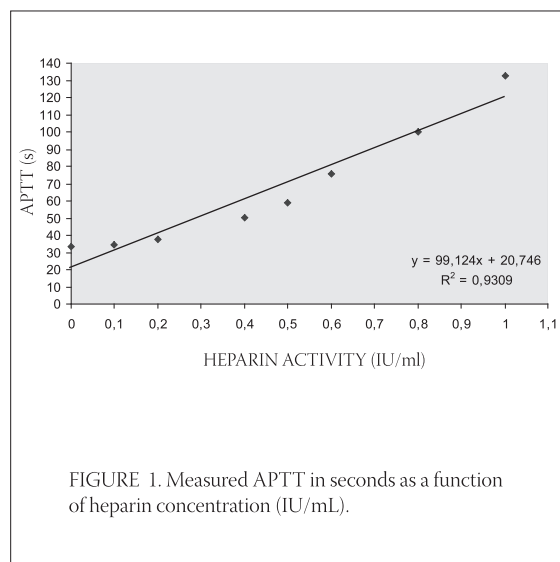


FIGURE 1. Measured APTT in seconds as a function of heparin concentration (IU/mL).

The linear regression line between 0,5 and 1,0 IU/mL of heparin concentrations had coefficient of correlation 0,995, that was acceptable for comparison of influence of FVIII:C.

## RESULTS AND DISCUSSION

Added amounts of FVIII:C of 1,0; 2,0; 3,0; 4,0 and 5,0 IU to NCCP (containing 0,98 IU/mL of FVIII:C) reduced APTT, as shown in Table 1. One way ANOVA-test, confirmed significant ( $p < 0,001$ ) reduction in basal APTT. Post-hoc Tukey-test of multiple comparisons showed that there were no significant changes in APTT between increased amount of spiked FVIII:C of 4,0 IU/mL and 5,0 IU/mL (25,27s vs 24,54 s;  $p = 0,078$ ). The influence of increasing amount of spiked FVIII:C on APTT in a presence of constant amount of heparin sodium is shown in Table 2. One way ANOVA-test showed significant ( $p < 0,001$ ) difference in APTT in simulated in vitro increase of FVIII:C activity in commercial plasma from 1,0 to 5,0 IU/mL, returning to the basal value with amount of spiked FVIII:C of 4,0 and 5,0 IU/mL. Tukey's test of multiple comparisons detected insignificant difference in reduced APTT values between increased amount of spiked FVIII:C of 1,0 IU/mL and 2,0 IU/mL (43,72 s vs 42,27 s;  $p = 0,344$ ), as well as between

SPIKED AMOUNT OF FVIII:C (IU/mL)	ACTIVITY OF FVIII:C (IU/mL) in NCCP	BASAL APTT (s) (0,98 IU/mL FVIII:C)	MEASURED APTT (s)
1,0	1,98	33,55	28,76
2,0	2,98	33,55	27,06
3,0	3,98	33,55	26,27
4,0	4,98	33,55	25,27
5,0	5,98	33,55	24,54

TABLE 1. APTT values as a function of increasing amount of spiked FVIII:C

HEPARIN SODIUM (IU/mL)	FVIII:C (IU/mL)	THEORETICAL ACTIVITY OF FVIII:C (IU/mL)	APTT (s)
0,5	0	0,978	58,88
0,5	1,0	1,978	43,72
0,5	2,0	2,978	42,27
0,5	3,0	3,978	37,40
0,5	4,0	4,978	34,89
0,5	5,0	5,978	33,72

TABLE 2. APTT values as a function of increasing amount of spiked FVIII:C in a presence of constant amount of 0,5 IU/mL of heparin sodium

HEPARIN SODIUM (IU/mL)	APTT (s) OF PLASMA (FVIII:C 0,98 IU/mL)	APTT (s) OF PLASMA (VIII:C 2,98 IU/mL)
0,5	58,55	42,27
0,6	76,05	43,72
0,8	99,71	57,90
1,0	132,81	73,76

TABLE 3. APTT values of commercial plasma (normal FVIII:C activity and spiked with 2,0 IU/mL) in presence of 0,5; 0,6; 0,8 and 1,0 IU/mL heparin sodium

4,0 IU/mL and 5,0 IU/mL (34,89 s vs 33,72 s;  $p = 0,586$ ). It was decided to further investigate how the variation in heparin concentrations, at fixed VIII:C (0,98 IU/mL spiked with 2,0 IU/mL) activity, influence on APTT values. The results are presented in Table 3. One way ANOVA-test showed significant ( $p < 0,001$ ) difference in APTT in simulated in vitro increase of FVIII:C activity in commercial plasma from 1,0 to 5,0 IU/mL, returning to the basal value with amount of spiked FVIII:C of 4,0 and 5,0 IU/mL. Tukey's test of multiple comparisons detected insignificance difference in reduced APTT values between increased amount of spiked FVIII:C of 1,0 IU/mL and 2,0 IU/mL (43,72 s vs 42,27 s;  $p = 0,344$ ), as well as between 4,0 IU/mL and 5,0 IU/mL (34,89 s vs 33,72 s;  $p = 0,586$ ). It was decided to further investigate how the variation in heparin concentrations, at fixed VIII:C (0,98 IU/mL spiked with 2,0 IU/mL) activity, influence on APTT values. The results are presented in Table 3.

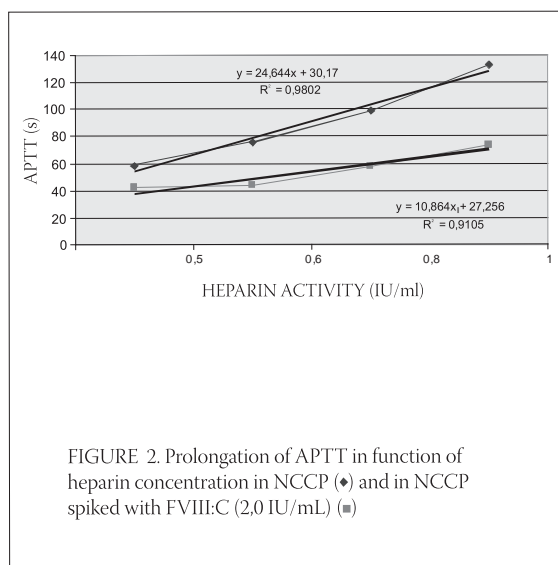


FIGURE 2. Prolongation of APTT in function of heparin concentration in NCCP (◆) and in NCCP spiked with FVIII:C (2,0 IU/mL) (■)

## CONCLUSION

As opposed to chemical spiking, biological spiking can be one very risky step, requiring appropriate piloting of the study. In this work we demonstrated the possibility for spiking of FVIII:C with plasma in one controlled way, giving possibility for further transfer of the method in clinical environment. The simple in vitro modeling of relation between F VIII:C concentration and heparin induced prolongation of APTT, primarily solved technical and mathematical aspects for the clinical ex vivo modeling.

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