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RESEARCH PAPER

Application of Adaptive DP-optimality to Design a Pilot Study for a Clotting Time Test for Enoxaparin

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ABSTRACT

Purpose Dosing of enoxaparin, like other anticoagulants, may result in bleeding following excessive doses and clot formation if the dose is too low. We recently showed that a factor Xa based clotting time test could potentially assess the effect of enoxaparin on the clotting system. However, the test did not perform well in subsequent individuals and effectiveness of an exogenous phospholipid, Actin FS, in reducing the variability in the clotting time was assessed. The aim of this work was to conduct an adaptive pilot study to determine the range of concentrations of Xa and Actin FS to take forward into a proof-of-concept study.

Methods A nonlinear parametric function was developed to describe the response surface over the factors of interest. An adaptive method was used to estimate the parameters using a D-optimal design criterion. In order to provide a reasonable probability of observing a success of the clotting time test, a P-optimal design criterion was incorporated using a loss function to describe the hybrid DP-optimality.

Results The use of adaptive DP-optimality method resulted in an efficient estimation of model parameters using data from only 6 healthy volunteers. The use of response surface modelling identified a range of sets of Xa and Actin FS concentrations, any of which could be used for the proof-of-concept study.

Conclusions This study shows that parsimonious adaptive DP-optimal designs may provide both precise parameter estimates for response surface modelling as well as clinical confidence in the potential benefits of the study.

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KEY WORDS Adaptive design · DP-optimal design · Response surface modelling · enoxaparin · factor Xa

ABBREVIATIONS

ACS	Acute coronary syndrome
aPTT	Activated partial thromboplastin time
AT	Antithrombin
BSV	Between subject variability
CT	Clotting time
DVT	Deep vein thrombosis
LMWH	Low molecular weight heparin
PE	Pulmonary embolism
PT	Prothrombin time
RE	Relative error
TenaCT	Ten-a clotting time
TT	Thrombin time
UFH	Unfractionated heparin
VTE	Venous thromboembolism

VARIABLES AND SYMBOLS

g	Unit of acceleration
j	Index for a parameter
$\mathbf{J}$	Jacobian matrix
k	Index for iteration
K_D	Affinity constant
$\mathbf{M_F}$	Fisher information matrix

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$\lvert\mathbf{M_F}(\cdot)\rvert$	Determinant of the Fisher information matrix
p	Total number of parameters
T	Transpose
U	Utility function
$\boldsymbol{\xi}_0$	Initial design
$\boldsymbol{\xi}_{\mathbf{D}}^{*}$	D-optimal design
$\boldsymbol{\xi}_{\mathbf{DP}}^{*}$	DP-optimal design
β	Any parameter in a model
$\widehat{\beta}$	Estimate of any parameter in a model
$\overline{\beta}$	Mean of any parameter in a model
$\boldsymbol{\beta}$	Vector of parameters in the model
$\widehat{\boldsymbol{\beta}}$	Vector of the estimated parameters
$\boldsymbol{\Sigma}$	Covariance of the residual error

INTRODUCTION

Prothrombin time (PT, usually reported as international normalized ratio) and activated partial thromboplastin time (aPTT) tests are used to monitor the anticoagulant effects of warfarin and unfractionated heparin (UFH), respectively. These clotting time (CT) tests have been shown to provide a good prediction of bleeding and thrombosis when used with warfarin and UFH. They are sensitive to deficiencies in the clotting network as well as the influence of anticoagulants and hence provide information on the overall clot forming potential of the patient. The need for a multi-step CT test for low molecular weight heparins (LMWHs) has been discussed previously and a factor Xa based CT (Ten-a clotting time, TenaCT) test has been shown to have the potential to be used for assessing the effect of enoxaparin, a LMWH anticoagulant, on the clotting system (1). The TenaCT test is generic to all LMWHs but enoxaparin was used as the example here. The findings were based on *in silico* simulations and *in vitro* experiments using plasma from a single healthy volunteer and provided supportive information for a proof-of-mechanism.

However, the TenaCT test provided extremely variable results in a subsequent group of individuals (pre-pilot study). One source of heterogeneity in CT tests is the presence of variable amounts of endogenous phospholipids which are important for the function of various clotting factors and complexes, *e.g.* tenase and prothombinase complexes. Various exogenous phospholipids were evaluated to minimize the variability observed in the pre-pilot study. A mixture of Actin FS with Xa was found to be effective in reducing the variability in CTs among individuals (results not shown). The concentrations of Actin FS and Xa appropriate for a reliable test are unknown. Their determination is a critical step in determining the experimental conditions under which the performance of the TenaCT test could be assessed in a proposed proof-of-concept study. Xa and Actin FS were therefore considered as design variables, only a single combination of which was possible to be used per individual. In addition, there was a limit of 6 individuals that could be enrolled for the pilot study due to prior ethical constraints. Figure 1 shows a schematic describing the previously conducted studies, present study and proposed future studies.

In this work, an adaptive design method is used in which the experimenter continually combines the information gained at each iteration of the experiment with the previously available information to update the design to be used for the next individual. In other words, the accrued data is used to "fine-tune" the experiment as it is being run. The adaptive design used here has similarities to methods that have been used to identify the minimum effective dose and maximum tolerated dose in early phase clinical trials and also to determine dose levels for the next phase clinical trials (2–4). Adaptive design and D-optimality have also been linked and shown to result in more accurate parameter estimates for the target population in bridging studies (5).

AIM

The overall goal of this pilot study was to find the range of concentrations of Xa and Actin FS that would provide the experimental conditions from which a planned future study could be conducted. The study conditions are intended to provide the highest probability of experimental success, *i.e.* the ability to assess the utility of the TenaCT. The specific objectives linked to the overall aim are:

(1) To develop a parametric function that describes the response surface (clotting time) following activation with Xa and Actin FS.
(2) To set up an adaptive design for updating parameters of the response surface function.

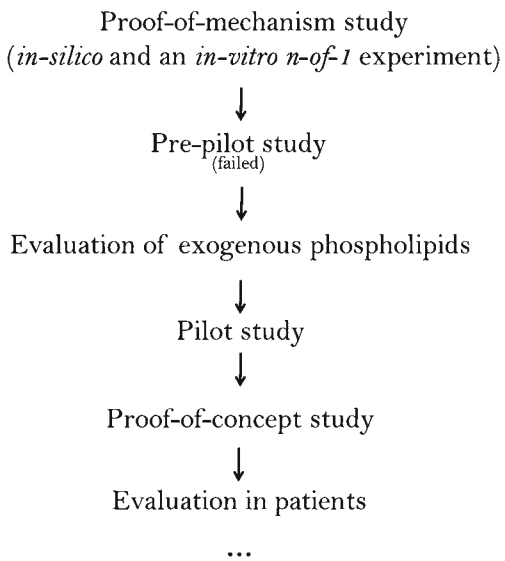

Fig. 1 A schematic describing the previously conducted studies, present study and proposed future studies for the development of the TenaCT test.

(3) To assess the performance of the pilot study using simulations.
(4) To perform the pilot study.
(5) To determine the study conditions appropriate for a future proof-of-concept study.

In carrying out the *in vitro* experiments with the 6 volunteers (specific objective 4), the design method from a simulation study (specific objective 3) would be followed, showing the implementation of the feasibility analysis discussed in the simulation study. The findings from the simulation study would guide the design of the actual pilot study that will contribute to proof-of-concept.

METHODS

Developing an Empirical Parametric Function to Describe the Response Surface (Clotting Time) Following Activation with Xa and Actin FS

Several models were evaluated to describe the relationship between Xa and CT and the effects of enoxaparin and Actin FS. Among the functions evaluated (linear, logarithmic, quadratic, exponential, power, E_{max}), the function described below was found to result in clotting times that aligned closely with a previously published blood coagulation systems pharmacology model (1,6) and to the results obtained in the preliminary *in vitro* experiments (data not shown).

$$CT = \left(\frac{(f_{ActinFS})^2}{(f_{Enox})^5} \times a \right) \times (f_{Xa})^b \quad (1)$$

The parameters a and b describe the relationship between Xa, Actin FS, enoxaparin and CT (in seconds). f_{Xa} is the unbound fraction of Xa available to form a clot and was described by:

$$f_{Xa} = 1 - \frac{[Xa]}{\left(\frac{f_{Enox}}{f_{ActinFS}} \times K_D^{Xa} \right) + [Xa]} \quad (2)$$

The parameter K_D^{Xa} describes the affinity of antithrombin (AT) for Xa in the absence of enoxaparin. Because enoxaparin produces its major anticoagulant effect by increasing the activity of AT against Xa, a coefficient f_{Enox} that describes the increase in the affinity of AT for Xa in the presence of enoxaparin was incorporated. As the concentration of enoxaparin increases the CT value would increase. In contrast, as the concentration of phospholipid increases the CT will decrease. The coefficient $f_{ActinFS}$ describes the decrease in the affinity of AT for Xa in the presence of phospholipid Actin FS. The coefficients were described by:

$$f_{Enox} = 1 - \frac{[Enox]}{K_D^{Enox} + [Enox]} \quad (3)$$

and

$$f_{ActinFS} = 1 - \frac{[ActinFS]}{K_D^{ActinFS} + [ActinFS]} \quad (4)$$

where K_D^{Enox} and $K_D^{ActinFS}$ are the parameters that describe the overall affinities of enoxaparin and Actin FS to Xa, respectively. The symbol "[ ]" in the above equations refers to the concentration of the species. The parameters that were to be estimated in this study were a, K_D^{Enox}, b and $K_D^{ActinFS}$. The value of K_D^{Xa} was fixed to 200 nM as per (7). The prior values for these fixed effect parameters are provided in Table I. These values were taken as starting points for the calibration so that the response surface provided similar clotting times to the coagulation systems model published previously (1,6) as well as to the preliminary *in vitro* experiments. However, it should be noted that this empirical function may not be appropriate for use outside of this particular study.

Setting Up an Adaptive Design for Updating Parameters of the Response Surface Function

CT experiments with Xa and Actin FS were to be performed using an adaptive design method (Fig. 2). In our approach to this design method, subjects are enrolled and their CT data is analysed sequentially. As more subjects are enrolled, their data are pooled with the data from previous subjects, along with their design variables and the model parameters re-estimated. Therefore in this study, iteration 1 consisted of data from volunteer 1;

Table I Prior Values for the Parameters Used in the Simulation Study

Parameter	Values
Fixed effects	
a	32
K_D^{Enox}	3.0 IU/mL
b	1.0
$K_D^{ActinFS}$	2.5%
K_D^{Xa}	200 nM (fixed)
Random effects (CV%)	
BSV_a	1%
BSV_K_D^{Enox}	10%
BSV_b	10%
BSV_$K_D^{ActinFS}$	10%
Residual (additive) error	1%

BSV between subject variability

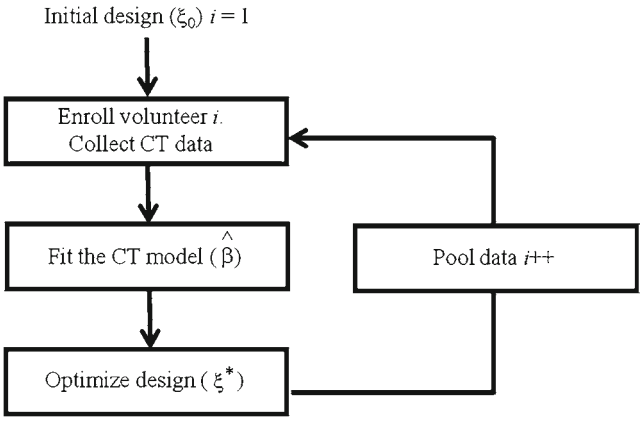

Fig. 2 A schematic of the adaptive design method.

iteration 2 consisted of pooled data from volunteers 1 and 2; and so on. After each iteration, the re-estimated parameters were used to optimize the design variables and the optimized values (ξ^*) were used to carry out experiments for the next volunteer. In this study, the controllable design variables (ξ) were concentrations of Xa and Actin FS and only a single combination of the two was permissible per volunteer. Each step of the adaptive method is explained below in detail in regards to the way it was used in this study.

Enrolling Volunteer and Collecting Data

The 1st volunteer was enrolled and CT determined at 4 concentration values of enoxaparin of 0, 0.25, 0.50 and 1.0 IU/mL. For the 1st volunteer, the concentrations of the activating agent Xa and the co-factor Actin FS (initial design, ξ_0) were equal to 20 nM and 1%, respectively. These values were based on the results obtained with the pre-pilot study.

Fitting the CT Model and Parameter Estimation

The CT model that described the relationship between CT, Xa, enoxaparin and Actin FS was fitted to the CT data obtained and parameters estimated. The parameter vector β was given by $[a, K_D^{Enox}, b, K_D^{ActinFS}]$. Parameter estimation was carried out in MATLAB (R2012a) using simulated annealing, with convergence given when the parameter estimates were significant to 3 digits. The concentrations of the activating agent Xa and co-factor Actin FS were set either at the value of the initial design for the first individual, or to the values that were previously optimized for subsequent individuals.

Optimizing Design

The estimated parameters obtained were used as the basis for optimizing the design variables, concentrations of Xa and Actin FS. Optimality was based on maximizing the precision of parameter estimation and maximizing the probability of experimental success according to the pre-defined criteria for a clotting time test described in the next section. A compound criterion was used where D-optimality (to provide precise parameter estimates) was combined with P-optimality (to provide a design that maximizes the probability that the CT values will meet the desired conditions). The criterion that DP-optimal design maximized was given by:

$$\boldsymbol{\xi}^*_{\mathbf{DP}} = \underset{\xi \in \Xi}{\operatorname{argmax}} \left(\left| \mathbf{M_F}\left(\widehat{\boldsymbol{\beta}}, \boldsymbol{\xi}, [Enox]\right) \right|^{1/p} U \right) \tag{5}$$

where $\boldsymbol{\xi}^*_{\mathbf{DP}}$ is the DP-optimal design concentration values for Xa and Actin FS, $\mathbf{M_F}$ is the Fisher information matrix, $|\mathbf{M_F}(\cdot)|$ is the determinant of the Fisher information matrix, $\widehat{\boldsymbol{\beta}}$ is the vector of the estimated parameters (from the previous iteration), p is the number of parameters and U is a utility function that was used to incorporate experimental success. $\mathbf{M_F}$ is equal to $\mathbf{J}^{\mathrm{T}}\Sigma^{-1}\mathbf{J}$, where "T" denotes the transpose of the matrix and $\boldsymbol{\Sigma}$ is the covariance of the residual error. $\mathbf{J}$ is a matrix of first partial derivatives of the model with respect to the estimated parameters, such that

$$\mathbf{J} = \begin{bmatrix} \frac{\partial f(Xa_1, ActinFS_1)}{\partial \widehat{\beta}_1} & \frac{\partial f(Xa_1, ActinFS_1)}{\partial \widehat{\beta}_2} & \frac{\partial f(Xa_1, ActinFS_1)}{\partial \widehat{\beta}_3} & \frac{\partial f(Xa_1, ActinFS_1)}{\partial \widehat{\beta}_4} \\ \vdots & \vdots & \vdots & \vdots \\ \frac{\partial f(Xa_n, ActinFS_n)}{\partial \widehat{\beta}_1} & \frac{\partial f(Xa_n, ActinFS_n)}{\partial \widehat{\beta}_2} & \frac{\partial f(Xa_n, ActinFS_n)}{\partial \widehat{\beta}_3} & \frac{\partial f(Xa_n, ActinFS_n)}{\partial \widehat{\beta}_4} \end{bmatrix}. \tag{6}$$

Note the D-optimal design considered fixed effects parameters only. The utility function U was equal to 0.9 when the criterion, described in the next section, was met for a successful CT test. It was equal to 0.1 when the criterion was not met, according to

$$U = \begin{cases} 0.9, \text{when CT is a success} \\ 0.1, \text{when CT is a failure} \end{cases}. \tag{7}$$

The choice of this optimality criterion (U=0.9 for success and 0.1 for failure) was chosen empirically. A value for success

to be 90% or greater was considered reasonable. For failure, however, a low value was assumed and 10% was considered reasonable. Different weighting structures could be considered but were not considered in this work. In initial calibration work, different values for success were considered in an exploratory fashion. The values chosen here reflect the current work only and are not intended to be considered for other adaptive optimal design applications.

The DP-optimality criterion was linked to an exchange algorithm for search across the design space (15-25 nM for Xa and 0.8-5% for Actin FS). The design space was described as a discrete interval for both design variables. For Xa the increment was 1 nM and for Actin FS the increment was 0.1%. This design space was chosen as it provided similar clotting times to the coagulation systems model published previously (1,6), based on the parametric function and the parameter values that were chosen. In addition, the function resulted in clotting times observed with the healthy volunteers in the preliminary *in vitro* experiments (data not shown). Any other combination of parameter values and design space could have been chosen too. DP-optimality was carried out using MATLAB (R2012a) and the obtained DP-optimal Xa and Actin FS values were then used to carry out CT experiments for the next volunteer.

Criterion for a Successful TenaCT Test. The experimental success of the TenaCT test was evaluated using the following conditions. The conditions, though empirical, were developed based on what is known with the other clotting time tests and the requirement that CTs in the absence and presence of various LMWH concentrations must be reasonably able to be conducted in a busy clinical diagnostic laboratory (*i.e.* < 200 s). The criterion consisted of 5 conditions, all of which were required to be met in order for the TenaCT test to be considered acceptable and hence successful in a volunteer:

- The CT in the absence of enoxaparin must be between 10 and 30 s.
- The fold increase in CT in the presence of 0.25 IU/mL enoxaparin must be at least 1.3-fold greater than the control value (CT in the absence of enoxaparin).
- The fold increase in CT in the presence of 1.0 IU/mL enoxaparin must be less than 5-fold that of the control.
- The fold increase in CT in the presence of 1.0 IU/mL enoxaparin must be at least 2-fold greater than the fold increase in CT in the presence of 0.25 IU/mL enoxaparin.
- All CTs must be < 200 s.

Pooling Data

The next volunteer was enrolled and CT values determined. This CT data was collected using the concentrations of the activating agent Xa and the co-factor Actin FS that arose from the DP-optimal design ξ_{DP}^{*} from the previous volunteer. The obtained CT data (clotting times, and values of their design points, concentration of Xa and Actin FS) from this volunteer was then pooled with the CT data from previous volunteer(s). These pooled data and the design variables were then used and the process repeated. This adaptive design method was continued until data from all 6 volunteers was available.

Assessment of the Performance of the Pilot Study Using Stochastic Simulations-Estimations

A simulation study was performed to assess the performance of the adaptive approach in 6 volunteers before the actual *in vitro* study was carried out. The simulation study was conducted using the same methods as intended in the actual adaptive design. Briefly, the CT values over the design variables, Xa and Actin FS, were described by the parametric function in equations 1-4. An adaptive design approach was used to estimate the parameters from the function so that the surface of the CT response could be explored in the actual pilot study. The purpose of the simulation study was to assess the precision with which the parameters of the function could be estimated using an adaptive DP-optimality method with 6 volunteers.

One hundred virtual pilot studies, each consisting of 6 patients, were simulated and analysed as per the pilot study protocol. Parameter values for each individual were simulated from Table I which includes between subject and residual variability in the parameter values. The individual parameter values were log normally distributed and the values of the between subject and residual variability parameters were chosen arbitrarily.

Parameter estimation was carried out in MATLAB (R2012a) using a simulated annealing algorithm. Design optimization was carried out using an exchange algorithm over a discrete design space of both design variables, Xa and Actin FS. Assessment of the precision and accuracy of the parameter estimates were considered for both adaptive D-optimal as well as adaptive DP-optimal designs. For each iteration k, percentage relative error (%RE) was calculated using the following equation:

$$\%\mathrm{RE} = \frac{\widehat{\beta}_{jk} - \overline{\beta}_j}{\overline{\beta}_j} \times 100 \tag{8}$$

where $\widehat{\beta}_{jk}$ is the estimate for the j^{th} parameter at the k^{th} iteration and $\overline{\beta}_j$ is the nominal mean of the j^{th} parameter for all simulated virtual clinical studies.

The numbers of successes of a pre-defined criterion for a successful TenaCT test were also compared for D- and DP-optimal designs.

Performing the Pilot Study

The present scenario for carrying out the pilot study to evaluate the TenaCT test was designed using DP-optimality based on an adaptive method to ensure precise parameter estimation as well as to maximize the probability of a CT success. MATLAB code showing the implementation is provided as part of the supplementary information. Factor Xa (catalytic activity-71 nkat) for the study was obtained from Abacus ALS, Auckland, New Zealand, a supplier of Chromogenix products. Enoxaparin used was a Clexane® subcutaneous injection available commercially (2000 IU) and co-factor Actin FS was obtained from Siemens Healthcare Diagnostics Ltd, Auckland, New Zealand.

Recruitment of Volunteers and Blood Collection

The pilot study consisted of a small cohort of 6 healthy volunteers. Ethics approval was obtained from the University of Otago Human Ethics Committee (reference code: 10/147). Written informed consent was obtained from all the participants in this study. Six healthy male volunteers, aged between 24 and 48 years, with no bruising or bleeding symptoms were recruited for the pilot study. Exclusion criteria included:

- Any inflammatory condition as a result of physical injury, immunological disorder (eg sprain, asthma, arthritis, *etc.*) or an infection
- Taking any prescription medicines that could suppress or treat an inflammatory response or that are known to affect clotting or bleeding time.
- Recent visit to the dentist: fillings or descaling within 7 days; extractions, gingival and other invasive procedures-within 21 days.

Blood collection was done by trained phlebotomists at the Blood Collection Centre, Dunedin Hospital. The blood was collected in standard vacutainers containing trisodium citrate as an anticoagulant. The volume and date of blood collected were recorded. Blood samples were sent from the Blood Collection Centre to the Southern Community Laboratories, Dunedin Hospital where they were centrifuged at 2000×*g* for 20 min at 20°C to obtain plasma. This was repeated and platelet counting performed to make sure the samples were platelet-free.

In Vitro CT Experiments

In contrast to measuring the CTs by visual detection in the proof-of-mechanism study (1), timing of clot formation in the pilot study was measured using STart® Hemostasis Analyzer-a viscosity based detection system. This analyser is used commonly in clinical haematological laboratories to perform various CT tests such as PT, aPTT and thrombin time (TT). The instrument was accessed at the Southern Community Laboratories, Dunedin Hospital where the experiments for this study were carried out.

A PT test is carried out by incubating a mixture of equal volumes of the activating agent (thromboplastin) and $CaCl_2$ (25 mM) at 37°C. The mixture is then added to a pre-warmed plasma sample and the time taken for the clot to form is recorded. An aPTT, on the other hand, has an additional incubation step and phospholipid present as part of the activating agent. The type and concentration of phospholipid varies among the commercially available reagents. The method to carry out the TenaCT test was kept closer to the PT test, for the sole reason of the PT test being simpler compared to an aPTT test. *In vitro* CT experiments in this work were carried out using equal volumes (100 μL) of pre-warmed (37°C) human plasma, calcium chloride (25 mM) and activator solution. The activator solution consisted of equal volumes (50 μL) of each of Xa and Actin FS. The plasma sample obtained from each volunteer was split into different vials and known amounts of enoxaparin added (1 part of enoxaparin was added to 9 parts of plasma) to provide concentrations of 0, 0.25, 0.50 and 1.0 IU/mL. The effect of enoxaparin on the CT was expressed as a ratio to the control value (CT in the absence of enoxaparin). Each CT measurement was performed in duplicate and mean CT values determined.

Determination of the Conditions Needed for a Future Proof-of-Concept Study

Using the parameter estimates obtained after the 6th iteration of the clinical study, CT values were simulated for each of the four enoxaparin concentrations (0, 0.25, 0.50 and 1.0 IU/mL) over a wide range of Xa (1.5-69) and Actin FS (0.090-4.1) values therefore providing four surface plots (one for each concentration of enoxaparin). The conditions, described previously as part of the criterion for a successful TenaCT test, were assessed at each grid point on the 4 CT surfaces to provide a response surface that described the probability of success of the TenaCT test over the range of sets of Xa and Actin FS values. Success was considered to be a continuous variable rather than all or nothing and the sets of Xa and Actin FS values were graded such that 100% success was applied if all 5 conditions were met, 80% success for any 4 conditions, 60% for any 3 conditions, 40% success for any 2 conditions, 20% success for any single condition and 0% success if no conditions were met. This provided a final plot of success *versus* concentrations of factor Xa and co-factor Actin FS. From this plot, values of Xa and Actin FS can be chosen for incorporation into the future proof-of-concept study.

Table II Comparison of Simulated CTs (in Seconds) in the Absence or Presence of Enoxaparin (1.0 IU/mL) Using the Empirical Parametric Function Described in Equations 1-4 and the Coagulation Systems Model Published Previously (1)

[Xa] (nM)	[Enox] (IU/mL)	Simulated CTs (in seconds)	
		Using the parametric function described in Equations 1-4	Using the coagulation systems model published previously
20	0	30	28
	1.0	190	205
10	0	35	42
	1.0	240	260

RESULTS

Developing an Empirical Parametric Function to Describe the Response Surface (Clotting Time) Following Activation with Xa and Actin FS

Table II summarizes the simulated CTs obtained using the empirical parametric function described in equations 1-4 and the coagulation systems model published previously (6). The CTs obtained using the function agreed well with the CTs from the systems model and the function was therefore taken forward to the simulation study.

Setting up an Adaptive Design for Updating Parameters of the Response Surface Function

No results for this section (see methods only).

Assessment of the Performance of the Pilot Study Using Simulations

One hundred virtual pilot studies were simulated to assess the performance of the adaptive D-optimal as well as DP-optimal designs. The simulations indicated that all four parameters a, K_D^{Enox}, $b, K_D^{ActinFS}$ could not be estimated for the first few iterations which required some to be fixed initially. At each iteration, as more CT data got pooled with the previous data, more parameters were able to be estimated. Table III provides a summary of the parameters that were estimated or fixed at each iteration.

The order in which the parameters were estimated also seemed important. Trying to estimate b and $K_D^{ActinFS}$ in the first few iterations resulted in highly inaccurate estimates and the estimates did not improve with pooling of more data as more parameters were estimated in the later iterations. Both b and $K_D^{ActinFS}$ were therefore fixed for the first few iterations in order to estimate all 4 parameters precisely by the 6th iteration. The order of estimating a or K_D^{Enox} did not significantly affect the overall outcome. The %RE for the estimates of a, K_D^{Enox}, $b, K_D^{ActinFS}$ in each iteration are shown in Fig. 3a for adaptive D-optimal and in Fig. 3b for adaptive DP-optimal designs.

Overall for all four parameters a, K_D^{Enox}, $b, K_D^{ActinFS}$, the estimates became more precise as more pooled data became available. It is seen that the accuracy and precision of the estimates of values of a and b improved when all parameters were estimated and were worse at iterations 4 and 5. It is probable that the change in accuracy and precision of the estimate of a at iteration 4 is due to the influence of the addition of the estimation of the parameter b. In this case imprecision would be due to the limited information to be shared across all parameters and possible interactions between the parameters. We see a similar trend in iteration 5, with the addition of the parameter $K_D^{ActinFS}$. However when all parameters were estimated when all data were available (iteration 6) this is resolved. We see the %RE of the estimates of all the parameters was reduced with the pooling of all data. This was seen with both adaptive D- and DP-optimality which shows that the parameter values were estimated accurately and precisely by the 6th iteration.

Comparison of the number of times the criterion was successful with D- *versus* DP-optimality showed that out of a total of 100 virtual pilot studies carried out with the D-optimal design, it provided a successful criterion in 23% of virtual studies (a virtual pilot study was considered a success when more than 3 out of 6 volunteers met the criterion). In contrast,

Table III Summary of the Parameters that were Either Estimated (E) or Fixed (F) at Each Iteration in the Adaptive Design. Fixed Parameters are Shown with the Values they were Fixed at

Iteration	Simulated volunteers (pooled data)	Parameters—either estimated (E) or fixed (F) (values that were fixed)			
		a	K_D^{Enox}	b	$K_D^{ActinFS}$
1	1	E	F (3.0)	F (1.0)	F (2.5)
2	1,2	E	F (3.0)	F (1.0)	F (2.5)
3	1,2,3	E	E	F (1.0)	F (2.5)
4	1,2,3,4	E	E	E	F (2.5)
5	1,2,3,4,5	E	E	E	E
6	1,2,3,4,5,6	E	E	E	E

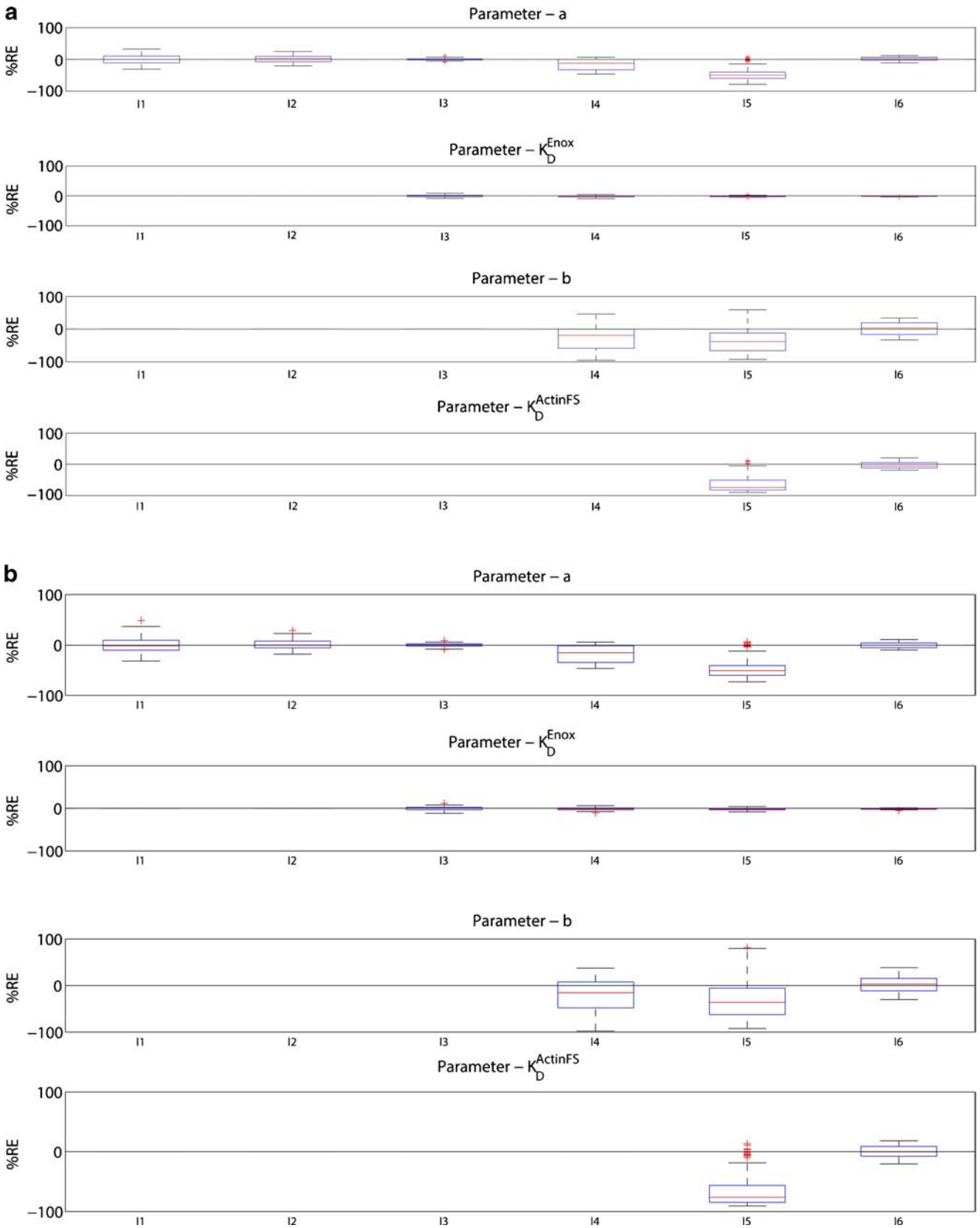

Fig. 3 Performance of adaptive design: Boxplots of percentage relative error (%RE) for estimates of the model parameters ($a, K_D^{Enox}, b, K_D^{ActinFS}$) obtained using an adaptive (**a**) D-optimal and (**b**) DP-optimal design method. The horizontal line within each subplot is the zero percentage difference from the nominal mean value. There were 6 iterations in each virtual pilot study which are shown as I1 to I6. For an iteration for which a parameter could not be estimated, a blank space is seen for that parameter at that particular iteration.

Table IV Parameter Estimates and DP-optimal Design Points Obtained Using the Adaptive Method in an Arbitrarily Chosen Virtual Pilot Study. The Parameter Values with an Asterisk (*) were the Values at which the Respective Parameters were Fixed

Iteration	Virtual volunteers (pooled simulated data)	Optimized design points		Parameter values			
		Xa	Actin FS	*a*	$KD\_^{Enox}$	*b*	$KD\_^{ActinFS}$
1	1	15	0.80	35	3.0*	1.0*	2.5*
2	1,2	17	0.80	38	3.0*	1.0*	2.5*
3	1,2,3	21	0.90	51	4.5	1.0*	2.5*
4	1,2,3,4	15	1.2	65	3.3	3.0	2.5*
5	1,2,3,4,5	15	0.95	43	2.5	7.2	19
6	1,2,3,4,5,6	15	0.85	41	2.1	13	4.1

it was found that DP-optimality provided a successful outcome in terms of the criterion in 94% of virtual studies. Table IV summarizes the parameter estimates and the DP-optimal design points obtained at each iteration of an arbitrarily chosen virtual pilot study.

Performing the Pilot Study

Enrolling Volunteer and Collecting Data

Activating the 1st volunteer's plasma using the initial design ξ_0 (20 nM Xa and 1.0% Actin FS) resulted in CTs that were measurable (<200 s) at tested enoxaparin concentrations (0, 0.25, 0.50 and 1.0 IU/mL). In addition, there was a reasonable and significant prolongation of CTs with increasing concentrations of enoxaparin. Table V shows the CTs obtained from the volunteers along with the criterion being a success or a failure for each volunteer. The results presented in this table were obtained after the application of the adaptive design, except in case of the 1st volunteer.

Fitting the CT Model and Parameter Estimation

The estimate for a single parameter *a* was obtained after fitting the model to the CT data from the 1st volunteer. This was part of the 1st iteration. In the following iterations, as more data were pooled with the previous data, more parameters were able to be estimated. Table VI summarizes the parameter estimates obtained at each iteration. Similar to Table V, the results presented in this table were obtained after the application of the adaptive design.

Optimizing Design

The DP-optimal design (ξ^*_{DP}) points after the 1st iteration were found to be equal to 15 and 0.80 for Xa and Actin FS, respectively. These design points were then used to carry out *in vitro* CT experiments for the 2nd volunteer. Table VI summarizes the DP-optimal design points obtained at each iteration.

Table V *In vitro* CT Experiments: Mean ± SD of Duplicate Measurements of CTs (in Seconds) in the Absence or Presence of Enoxaparin (0.25-1.0 IU/mL) for Plasma Obtained From Six Healthy Volunteers

Volunteer	Concentration used in the *in vitro* experiments		[Enox] 0 IU/mL	[Enox] 0.25 IU/mL		[Enox] 0.50 IU/mL		[Enox] 1.0 IU/mL		Criterion (success or failure)
	[Xa] (nM)	[Actin FS] (%)	Mean ± SD CT (seconds)	Mean ± SD CT (seconds)	Fold prolongation	Mean ± SD CT (seconds)	Fold prolongation	Mean ± SD CT (seconds)	Fold prolongation	
1	20	1.0	18 ± 0.4	24 ± 1.3	1.3	35 ± 0.8	2.0	57 ± 2.3	3.2	success
2	15	0.80	17 ± 0.6	38 ± 1.7	2.2	47 ± 0.3	2.7	62 ± 0.1	3.6	failure
3	15	0.80	17 ± 0.3	29 ± 0.6	1.7	42 ± 1.3	2.5	73 ± 0.2	4.3	success
4	15	0.80	21 ± 0.3	47 ± 0.1	2.2	65 ± 3.0	3.0	100 ± 3.0	4.9	success
5	15	0.80	16 ± 0.1	27 ± 0.2	1.6	38 ± 0.6	2.3	58 ± 0.4	3.5	success
6	15	3.3	17 ± 0.2	25 ± 0.2	1.5	33 ± 0.4	1.9	52 ± 0.5	3.1	success

SD standard deviation, obtained from the duplicate measurements for each plasma sample

Table VI Parameter Estimates and Optimized Design Points Obtained Using the Adaptive Method. The Parameter Values with an Asterisk (*) were the Values at which the Respective Parameters Were Fixed

Iteration	Volunteers (pooled data)	Optimized design points		Parameter values			
		Xa	Actin FS	*a*	$KD\_^{Enox}$	*b*	$KD\_^{ActinFS}$
1	1	15	0.80	31	3.0*	1.0*	2.5*
2	1,2	15	0.80	31	3.0*	1.0*	2.5*
3	1,2,3	15	0.80	42	4.2	1.0*	2.5*
4	1,2,3,4	15	0.80	74	3.2	10	2.5*
5	1,2,3,4,5	15	3.3	52	3.1	11	25
6	1,2,3,4,5,6	15	0.90	60	3.1	11	7.1

Pooling Data

In the 2nd iteration, *in vitro* CT experiments were carried out with the plasma from the 2nd volunteer using optimized concentrations of the design variables from the 1st volunteer. The CT data and the respective design variables were pooled across the 1st and the 2nd volunteers and used to estimate a single parameter *a*. The optimized design variables obtained using the estimated parameter values were again found to be equal to 15 and 0.80 for Xa and Actin FS, respectively. These optimized Xa and Actin FS values were used to carry out *in vitro* CT experiments with the 3rd volunteer. This was continued until volunteer 6.

Based on the pooled CT data and using adaptive DP-optimality to optimize the design variables, CTs in the absence of enoxaparin ranged from 16 to 21 s in the cohort of volunteers used in this set of experiments. A mean CT prolongation of 1.8±0.40 fold in the presence of 0.25 IU/mL enoxaparin and 3.8±0.70 fold when the enoxaparin concentration was 1.0 IU/mL was observed (see Table V). Using adaptive DP-optimality, concentrations of Xa and co-factor Actin FS reached values of 15 nM and 0.90% respectively by the 6th iteration and the criterion for a successful TenaCT test was met in 5 out of 6 healthy volunteers.

Simulation of the Response Surface

The parameter estimates obtained after the 6th iteration, $\hat{\beta} = \{60, 3.1, 11, 7.1\}$ were used to simulate the CT values using the parametric function described earlier in equations 1-4. The CT values were determined over a wide range of Xa (1.5-69) and Actin FS (0.090-4.1) values. Figure 4 shows the surface plots for each of the four matrices of CT values for the four enoxaparin concentrations (0, 0.25, 0.50, 1.0 IU/mL). Assessment of the obtained CT surfaces against the conditions for a successful TenaCT test resulted in a response surface that covered the range of sets of values of Xa and Actin FS determining the overall success of the TenaCT test. Figure 5 shows various regions of 100, 80, 60 and 40% success for the

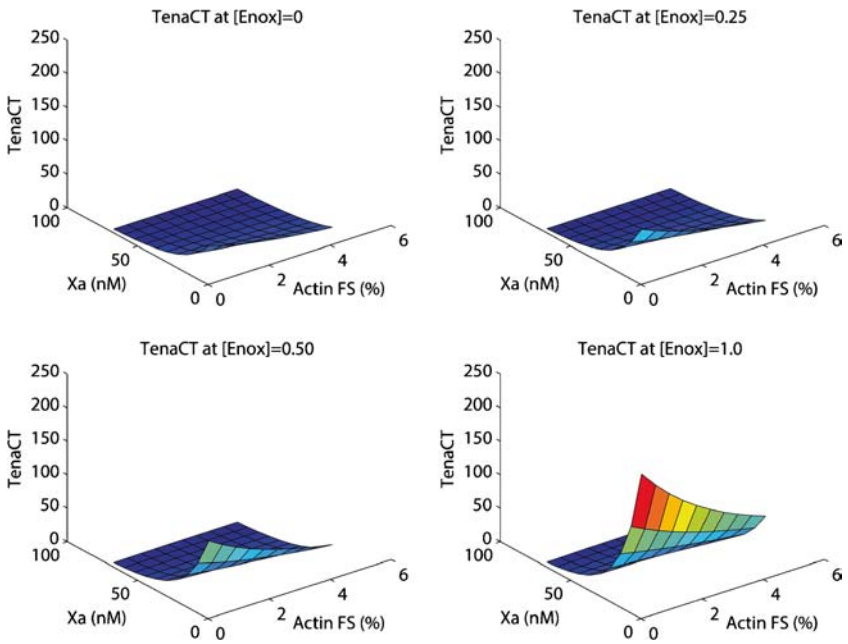

Fig. 4 Surface plots for each of the 4 matrices of simulated CT values at enoxaparin concentrations of 0, 0.25, 0.50 and 1.0 IU/mL.

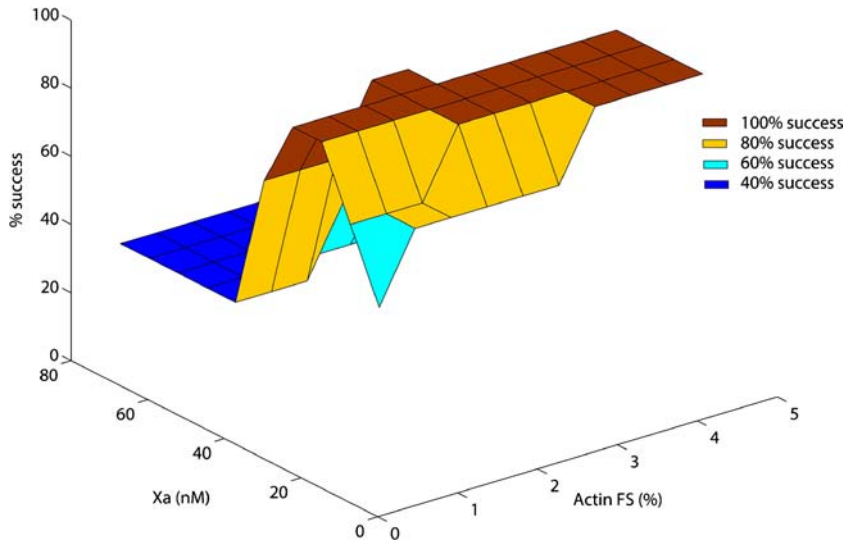

Fig. 5 Response surface covering the range of sets of values of Xa and Actin FS determining the overall success of the TenaCT test.

TenaCT test. The brown area in the plot shows the areas where all 5 conditions for a successful TenaCT test were met and therefore, denotes a 100% success. Table VII shows the range of Xa and Actin FS values that would give 100, 80, 60 and 40% success for the TenaCT test.

DISCUSSION

The simulation study and the actual pilot study in this work have shown application of an adaptive method, using DP-optimality, to design a pilot study to evaluate a clotting time test for assessing the anticoagulant effect of enoxaparin. The pilot study provided a range of sets of values of Xa and Actin FS that could be used for the future proof-of-concept study. These are a flexible set of conditions and using any of these would be expected to give a high probability of success of the proof-of-concept study. These sets of values were identified by evaluating the expected response over the values of the design variables (Xa and Actin FS) at the four enoxaparin concentrations to evaluate the overall success of the TenaCT test.

Table VII Ranges of Values of Xa and Actin FS that Would Give a Successful TenaCT test

Success	Sets of Xa and Actin FS values that would give a success	
	[Xa]	[Actin FS]
100%	1.5	2.8–4.1
	9.0	1.4–4.1
	17–24	0.090–4.1
	32	1.4–1.8
80%	1.5	0.54–2.3
	9.0	0.090–0.99
	32	0.090–0.99
	32	2.3–4.1
	39	3.2–4.1
60%	39	1.5–2.8
	47	3.2–4.1
40%	39	0.090–0.99
	47	0.090–2.8
	54–69	0.090–4.1

The simulation study used an adaptive method where parameters of the model were estimated based on the pooled data from virtual volunteers. At each iteration, data from a present virtual volunteer was combined with the data from previous volunteers to estimate the parameters. This provided precise estimation of all 4 parameters by the 6th iteration (consisted of pooled data from all 6 virtual volunteers). The design variables, concentrations of Xa and Actin FS, were optimized based on the estimates from the pooled data. Optimization of design variables was considered using D-optimality and DP-optimality separately. Both D-optimality and DP-optimality appeared to perform interchangeably with respect to precision of parameter estimates and both appeared to perform acceptably with 6 individuals. In contrast, DP-optimality provided a significantly greater proportion of studies that were considered successful in terms of clotting time goals compared to D-optimality.

With both D-and DP-optimal designs, it was seen that the estimates approached the nominal mean values of the parameter estimates as more data got accumulated. The simulations indicated that as more data became available, the initial biases in the parameters were reduced. It appeared that the parameter estimates were not biased and were acceptably precise for the 6th iteration which suggested that 6 volunteers would be sufficient to estimate the parameter values of the model using this adaptive design technique. Importantly, the simulations

identified that not all parameters could be estimated from a single patient's data (or even after several patient's data had been collected) and provided evidence supporting which parameters should be estimated initially.

The use of an adaptive D-optimal design obtained by maximizing the information based on the updated estimates would in theory ensure accurate estimation at the final iteration. However, with D-optimality it may not be possible to determine if the Xa and Actin FS values chosen would work in the proposed *in vitro* set up. Therefore, a D-optimal design may not provide clinical confidence of a success for a future clotting time study based on empirical observation of the current study results. However, using DP-optimality, which provided a predicted 94% chance of success in the simulation study also provided empirical evidence of success in the actual study with 5 of the 6 volunteers meeting the criteria. This provides clinical confidence for future studies. Importantly, statistical confidence was gained as the DP-optimality parameter estimates were not obviously worse (less precise or more biased) than for D-optimality.

It was noted in the adaptive design that the values of Xa did not change from 15 nM which was considered to be due to the narrow permissible range over which its activity provides experimental success. Of note, we saw the same outcomes in both the simulation study and the actual study where the value of Xa either started or ended up at 15 nM and was not apparently adapted after this value. A broader range of initial starting values would have shown a greater influence of the adaptive process.

The obtained parameter estimates at the end of 6 iterations from the actual study were used to simulate CT surfaces at the 4 enoxaparin concentrations of 0, 0.25, 0.50 and 1.0 IU/mL. It could be seen from the surface plots in Fig. 4 that the CTs were longer in the presence of higher enoxaparin concentrations, shorter in the presence of higher Actin FS concentrations and shorter, again, in the presence of higher Xa concentrations. Similar trends have been observed with other clotting factors, anticoagulants and clotting time tests (8).

Assessment of the 4 CT surfaces against the pre-defined conditions for a successful TenaCT test resulted in a range of concentrations of Xa and Actin FS that are likely to provide a successful proof-of-concept study for the TenaCT test. Interestingly, any Xa values within the range of 17-24 nM could be used with any Actin FS value between 0.090-4.1% to get a 100% success (all 5 conditions will be met). Overall, the use of adaptive method along with a compound criterion resulted in an efficient estimation of model parameters using data from only 6 healthy volunteers. The use of response surface modelling identified a range of sets of Xa and Actin FS concentrations, any of which could be used for the proof-of-concept study.

This study should not be used to imply that only 6 patients would be required for a clotting time (or similar) study. We would recommend that a simulation study is set up to assess the performance of an adaptive design method to ensure the maximum design constraints, number of patients, admissible design space, and proposed response surface model are likely to provide designs that are stable and work as intended. This study does show that parsimonious adaptive DP-optimal designs may provide both precise parameter estimates for response surface modelling as well as clinical confidence in the potential benefits of the study.

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