Arteriosclerosis, Thrombosis, and Vascular Biology

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Series Editor: Kellie R. Machlus

Computationally Driven Discovery in Coagulation

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ABSTRACT: Bleeding frequency and severity within clinical categories of hemophilia A are highly variable and the origin of this variation is unknown. Solving this mystery in coagulation requires the generation and analysis of large data sets comprised of experimental outputs or patient samples, both of which are subject to limited availability. In this review, we describe how a computationally driven approach bypasses such limitations by generating large synthetic patient data sets. These data sets were created with a mechanistic mathematical model, by varying the model inputs, clotting factor, and inhibitor concentrations, within normal physiological ranges. Specific mathematical metrics were chosen from the model output, used as a surrogate measure for bleeding severity, and statistically analyzed for further exploration and hypothesis generation. We highlight results from our recent study that employed this computationally driven approach to identify FV (factor V) as a key modifier of thrombin generation in mild to moderate hemophilia A, which was confirmed with complementary experimental assays. The mathematical model was used further to propose a potential mechanism for these observations whereby thrombin generation is rescued in FVIII-deficient plasma due to reduced substrate competition between FV and FVIII for FXa (activated factor X).

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: algorithms ■ hemophilia A ■ machine learning ■ plasma ■ thrombin

This review highlights our recent study in which we used computational modeling to discover a potential modifier of thrombin generation in mild to moderate hemophilia A.[1] We were motivated by the variation in bleeding frequency and severity within clinical categories—severe, moderate, mild—of hemophilia A,[2,3] characterized by coagulation FVIII (factor VIII) levels of <1%, 1% to 5%, and 5% to 20%, respectively. Although the bleeding severity in the majority of patients correlates with their factor levels, up to 10% of patients in the severe category have a bleeding phenotype that would be classified clinically as mild.[4,5] The underlying mechanism(s) of these observations are unknown. This prompts the question: in the case of severe quantitative defects with mild bleeding, what is compensating for the deficient clotting protein? If we can answer that question, we may identify variable(s) that can be adjusted or manipulated to improve outcomes for patients with high-frequency bleeding and, more generally, help predict bleeding patterns early in life, before serious bleeding begins. However, numerous variables could potentially be at play either working alone, additively, antagonistically, or synergistically. These variables could be biological, biochemical, or biophysical in nature. Measuring all possible variables in a clinical environment or testing how single variables affect the clotting response under flow, one at a time, would be time consuming and expensive, and in some cases, not possible. Our goal was to exploit the efficiency of a computationally driven approach to search for clues to solve this scientific mystery.

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For Sources of Funding and Disclosures, see page 85.

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Nonstandard Abbreviations and Acronyms

F	factor
TF	tissue factor

Highlights

- Computational approaches complement experimental ones and enable discovery in coagulation.
- Enormous synthetic data sets, created with computational models, can be used for focused and efficient searches for modifiers of coagulation.
- Iterating between mathematical predictions, experimental validation, and mathematical exploration of mechanism allowed for identification of FV (factor V) as a modifier of thrombin generation in mild to moderate hemophilia A.

Data-Driven Versus Computationally Driven Approaches to Discovery in Coagulation

Discovering these clues, particularly if multiple factors are involved, requires the generation and analysis of large data sets comprised of experimental outputs or patient samples. One approach to this problem is to simply look for patterns in such data sets with statistical or machine learning algorithms, an approach known as data-driven. In this case, algorithms are trained on existing data sets to learn features that are associated with well-defined outcomes. These approaches typically improve with exposure to more and more data and with good data sets and well-defined inputs and outputs; these approaches can yield useful predictive tools. For example, machine learning was used to inform dosing strategies for warfarin,[6–8] which is known to have a wide interindividual variability in dose requirement,[9] incorporating dozens of input characteristics for thousands of patients. Another example of this approach is from Chatterjee et al,[10] in which a data set was generated in vitro specifically to train a machine learning algorithm to learn platelet calcium responses, the ultimate goal being prediction of responses due to any combination of agonists beyond the training data. This method was extended to handle additional agonists to generate a human platelet calcium calculator[11] and used to predict platelet activation states within a large multiscale mathematical model of platelet deposition under flow.[12] Machine learning algorithms can identify patterns and serve as powerful prediction tools, but because of their nonmechanistic nature, they may not be able to determine the mechanism(s) underlying those patterns.

To address our question using a data-driven approach, we first needed to determine if generating an ideal experimental data set was even feasible. An ideal data set would involve systematic variation of clotting factor and inhibitor concentrations and subsequent monitoring of thrombin generation and thrombus formation under flow, for each variation. This can quickly require a very large number of complicated experiments, for example, choosing only 5 clotting factors to vary could result in over 2000 different possible combinations, plus technical replicates. Furthermore, unless a synthetic plasma is used for the experiments, modulating the levels of individual clotting factors in whole blood is challenging. Performing a handful of these experiments is certainly doable but generating the entire desired data set is not. This roadblock, combined with our goal to understand the mechanism(s) underlying the observed variability in bleeding severity, led us to take a different approach that we call computationally driven. We illustrate our computational driven framework in Figure 1.

What if instead of generating a large experimental data set, we use a mathematical model to generate a large computational data set? In such mathematical experiments, we can easily and systematically vary all of the clotting factor and inhibitor concentrations within a specified range. For each set of variations, we then run the model to produce a computational prediction of thrombin generation and clot formation under flow. This process allowed us to, in essence, create a mathematical depiction of thrombin generation in hundreds of thousands of individuals with moderate to mild hemophilia A, or in other words, create synthetic patient data. Generation of this type of data set is possible because of computational efficiency since one of the simulations takes only seconds to complete. We can generate a massive amount of data that can subsequently be statistically analyzed to extract and identify complex interactions between multiple clotting factors or inhibitors, as we illustrate in Figure 2. Moreover, with a mechanistic mathematical model, as further described below, we have access to the time-varying concentrations of every coagulation reactant and reaction intermediate, which enables us to search for biochemical mechanisms underlying any patterns found in the data. This means that, with our model, in addition to monitoring the changes that occur in the output (thrombin generation), we can determine how those changes occur.

To statistically analyze the synthetic patient data, we first need a mathematical metric to serve as a surrogate for bleeding severity and then need to study how that metric is affected by manipulations of model inputs. We chose thrombin generation as our surrogate metric of bleeding severity and clotting factor and inhibitor levels that are input into the mathematical model as potential biomarkers. Our choice of metric is suitable since thrombin generation is decreased in hemophilias A and B; deficiency in clotting factors VIII or IX leads to decreased thrombin generation since they comprise the tenase

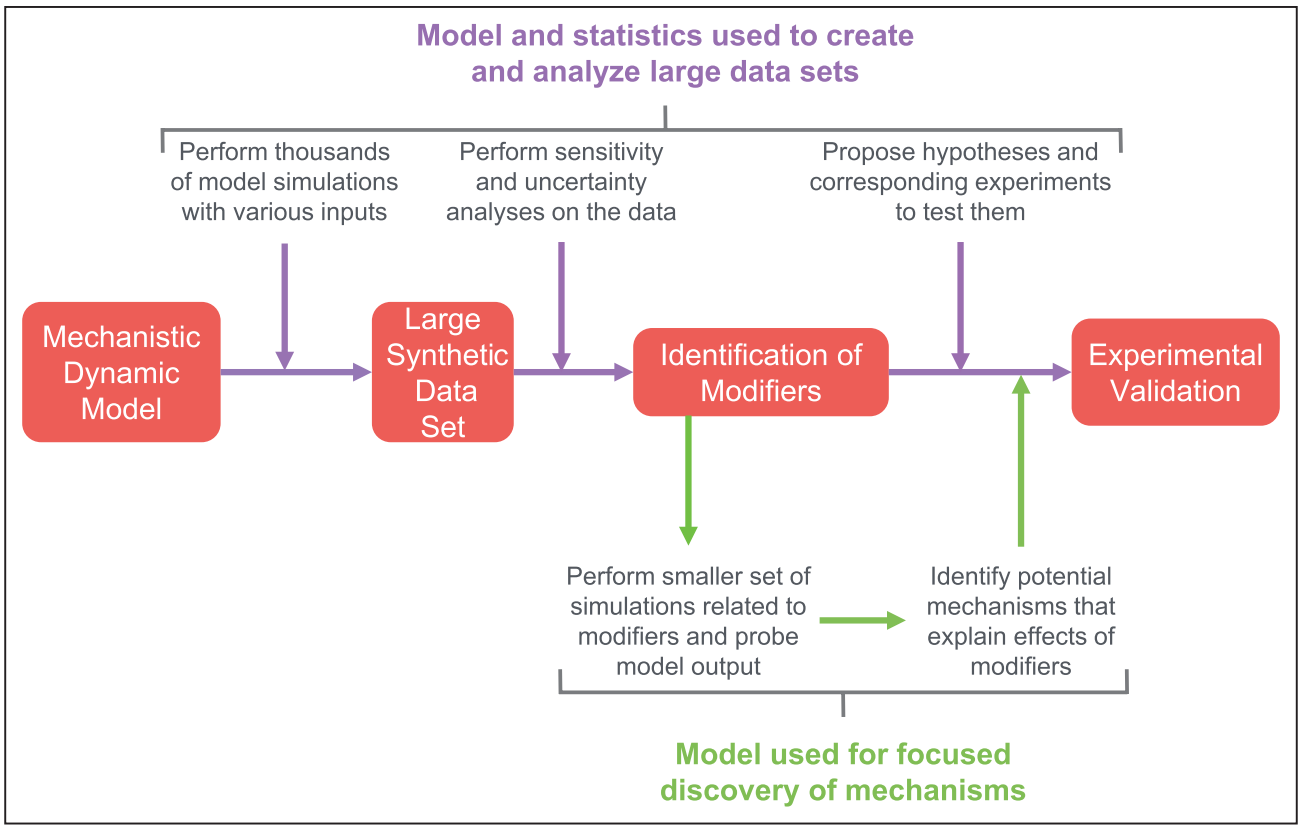

Figure 1. Computationally driven approach for discovery in coagulation.
A mechanistic mathematical model is used to create large synthetic patient samples which are then analyzed to reveal modifiers, propose mechanisms, and generate hypotheses for experimental validation.

complex, which is necessary for the propagation phase of coagulation and subsequent robust thrombin generation. We chose clotting factors and inhibitors as inputs since they are commonly measured and because, interestingly, their normal ranges are accepted as around 50% to 150% of the mean values of the healthy population.[13,14] Because of this known variation, we hypothesized there could be clues hidden within the plasma composition and that there may be synergistic or antagonistic behavior between proteins at levels still considered normal. To test this hypothesis, we asked the following question: how do clotting factor and inhibitor levels alter thrombin generation during flow-mediated coagulation when factor VIII is deficient? Investigating this question requires the use of a mechanistic dynamic model that describes flow-mediated coagulation. We next describe what we mean by dynamic models and briefly review some dynamic models of coagulation.

Dynamic Models and Coagulation Thresholds

A dynamic model of coagulation is a mathematical representation of a network of biochemical reactions that yields time-varying concentrations of coagulation proteins. A mechanistic dynamic model of coagulation is one that is based on explicit consideration of the reactions that yield observed thrombin generation data; an example of a nonmechanistic model of coagulation is a descriptive model that provides a quantitative summary of observed data, such as using linear regression to fit a line to data. The Mann laboratory developed one of the first dynamic models of the extrinsic blood coagulation system. That model displayed a nonlinear, threshold-like dependence of thrombin generation on concentrations of TF (tissue factor) bound to FVIIa, tissue factor pathway inhibitor, and antithrombin,[15,16] which agreed well with previous observations made in the authors' experimental studies using synthetic plasma.[17] In relation to our work described below, the Mann model was also used in a combined experimental and computational approach to investigate variation in thrombin generation in mild to moderate hemophilia A as a result of changing other variables in the model.[18] The authors simulated thrombin generation at various concentrations of FVIII with other factor levels set to either all high-normal (150% of mean values) or all low-normal levels (50% of mean values). Interestingly, their model showed that if all factor levels are at high-normal levels when FVIII is low, that the thrombin generation can be slightly enhanced. This model has been used extensively to investigate sensitivity to normal variations of clotting factors in healthy individuals,[19] to assess risk of disease,[20] and to investigate mechanisms underlying

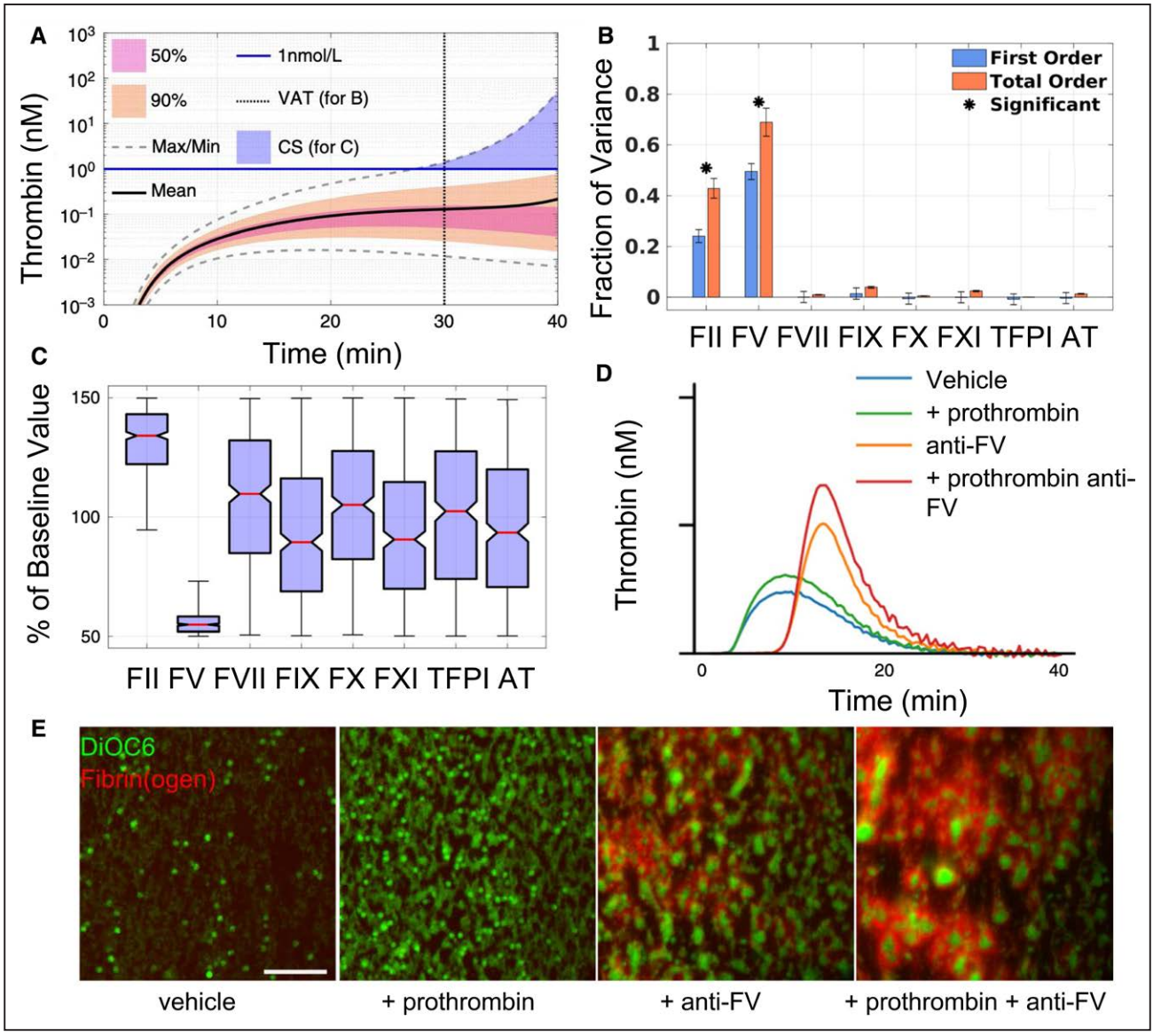

Figure 2. A mathematical model of coagulation under flow identifies FV (factor V) as a modifier of thrombin generation in hemophilia A.

FV is a modifier of thrombin generation in a mathematical model of flow-mediated coagulation. **A**, Thrombin concentration time series generated by uniformly and independently varying plasma protein levels including inhibitors tissue pathway inhibitor (TFPI) and antithrombin (AT) ±50% from normal (110 000 total simulations); mean (solid black line), boundaries that encompass 50% (pink), 90% of the data (orange), and the maximum/minimum of the computed solutions (gray-dashed); blue line drawn at 1 nM. **B**, First (blue) and total (orange) order Sobol indices are plotted as mean±SD computed with 5000 bootstrap samples of the original 110 000 simulations. **C**, Plasma protein levels distributions shown as box-and-whisker plots (mean in red, whiskers drawn at 3× the interquartile range), conditioned on achieving >1 nM of total thrombin by 40 min. **D**, Calibrated automated thrombography. FVIII-deficient (<10%) plasma treated with vehicle control, 50 µg/mL exogenous prothrombin, 100 µg/mL anti-FV, and exogenous prothrombin and anti-FV. Assay conducted with 5 pM TF (tissue factor) and phospholipids. **E**, Flow assays with whole blood from FVIII-deficient individuals. **A**, Representative images of DiOC6 labeled platelets and leukocytes and Alexa Fluor 555 labeled fibrin(ogen) on collagen-TF surfaces at 100 per second after 25 min for vehicle control, 50 µg/ mL exogeneous prothrombin, 100 µg/ mL anti-FV, and exogenous prothrombin and anti-FV. Scale bar=50 µm. CS indicates condition for samples; and VAT, variance analysis time.

complications of trauma and coagulopathies,[21,22] but it does not explicitly consider lipid surfaces, either phospholipid vesicles or activated platelet surfaces, known to regulate coagulation.[23–25] Collectively, these studies suggest that the type of surface, that is, lipid vesicle versus platelet surface, may be important in differentiating and understanding coagulation thresholds.[26] Additionally, biophysical effects on coagulation such as flow and diffusion likely contribute to how the thresholds manifest in vivo, but that cannot be studied with static models. We refer the reader interested in models of static coagulation to other reviews.[27–31]

Models incorporating the dynamic interplay between flow, surface, and coagulation allow for investigations

considering transport effects. Kuharsky and Fogelson[32] developed a model (denoted as KF model) to simulate thrombin generation at the site of a small vascular injury under flow. The model accounts for platelets, platelet deposition, and surface-mediated coagulation reactions, all under flow. Transport of each protein and platelet species to and from the reaction zone by flow and diffusion is described using mass transfer coefficients.[33] The reaction zone is a region located just above a small patch of exposed subendothelium; its height is defined by how high a protein molecule can be above the injury and still diffuse to the injury before being carried away by the flow. The authors proposed an inhibitory, anticoagulant role for the adherent platelets, namely that they physically inhibit the enzyme complexes embedded in the injury patch. This notion of paving over the injury was later confirmed experimentally.[34] The KF model was later extended to include endothelial cell reactions[35] and the FXI pathway.[36] Results from these models aided in proposing testable hypotheses including thrombin generation dependence on a surface tissue factor threshold under flow[32,35] (later experimentally validated at venous and arterial shear rates[37]); the effects of platelet count on thrombin generation under flow in hemophilia C[36]; and the effects of FXIa and TF in postintravenous immunoglobulin treatment of thrombotic events.[38] There are a variety of other models of clotting under flow, using different mathematical and computational techniques, including some spatial models that account for each individual red blood cell and platelet. Here, we base our studies on the extended version of the KF model but refer the reader to other reviews devoted to this topic.[39–47]

Quantifying Uncertainty in Computational Models

The extended KF model, as well as other models described above, rely on numerous assumptions regarding the biochemical reaction networks, kinetic rate constants, and the experimental assays used for model calibration. The inherent uncertainty in these assumptions raises an issue of the value of kinetic modeling of thrombin generation for prediction and proposal of testable hypothesis.[48,49] A main point of contention involves thrombin generation simulated with different existing mathematical models giving contrasting results, even when using the same initial concentrations of plasma proteins.[48] Counter arguments in favor of mathematical modeling stress that for a model to be truly predictive and accurately simulate coagulation reactions and perturbations; it must be carefully validated with the experiments that it simulates, and any additional sources of uncertainty and variability should be quantified. Statistical methods attempt to examine the extent to which model outputs depend on model inputs. Specifically, sensitivity analysis and uncertainty quantification methods seek to assess how the uncertainty in a model input (such as a kinetic rate or factor level) propagates through the model to produce variations in a model output (such as the level of thrombin at a particular time). Many uncertainty quantification approaches exist and, depending on the question under investigation, they may be employed to identify plausible ranges of model inputs from observations of model outputs or to investigate the variability, uncertainty, and goodness of a particular computational model itself.

Sensitivity analysis techniques have been used to study static and flow-mediated models of coagulation.[15,19,50–52] Danforth et al[50] performed a local sensitivity analysis of the Mann model[15] to investigate the sensitivity of thrombin generation as a function of its reaction rates, as well as to investigate the sensitivity of thrombin production to initial coagulation protein concentrations[19] alone or through their pairwise changes/interactions. A similar analysis was conducted by Naidu and Anand[51] on the model of Anand et al.[53] In Link et al,[54] a tailored sensitivity analysis approach was developed to analyze the effects of variability within the model of Fogelson et al.[36] In that approach, concentrations of coagulation proteins, kinetic rate constants, and biophysical and platelet specific parameters were varied. Robust thrombin production was seen for variations of plasma proteins within the physiological range, and wider variations in thrombin generation occurred with variation in individual kinetic rate constants.

Identifying FV as a Modifier of Thrombin Generation in Hemophilia A Using a Computationally Driven Approach

Using our mathematical model[36] and computationally driven approach, we generated an enormous amount of synthetic patient data and used it to identify FV as a modifier of thrombin generation in mild to moderate hemophilia A. All simulations used to generate the data set considered a low TF density of 5 fmol/cm^2 and a low shear rate of 100 s^{-1}. Motivated by our previous studies,[32,35,38] we first did an initial screening and determined a range of critical TF levels where thrombin sharply transitioned between an attenuated response with thrombin peaking at a concentration below 1 nmol/L and an amplified thrombin response in which the thrombin concentration reached at least 1 nmol/L by 40 minutes; the TF range is 4.63 to 7.78 fmol/cm^2 for FVIII-deficient plasma. Since individuals with FVIII deficiencies are known to bleed in regions of the body with low TF levels,[55–57] we chose a single TF level, 5 fmol/cm^2, near the low end of our computationally determined critical TF range.

Our synthetic patient data set exhibited large variance in thrombin generation due to changes in clotting factor and inhibitor levels, see Figure 2A. To determine

which clotting factor and inhibitor levels were the most important for enhancing thrombin generation within our synthetic data set, we used Sobol sensitivity analysis[58] to determine the fraction of the observed variance in thrombin generation that was attributable to each factor or inhibitor level, see Figure 2B. We found that FV and prothrombin (FII) contributed the most to the variance, with their interaction explaining nearly 20% of it. We then looked more directly at the ≈5% of simulations that produced an amplified thrombin response (see above) in FVIII-deficient plasma. We examined the plasma clotting factor and inhibitor levels that produced the amplified response and observed that the distributions of these levels were highly skewed for 2 factors: FV and prothrombin. All simulations with an amplified response had plasma FV level of <75% of its mean population level and most had a prothrombin level above 125% of its mean population level (shown in Figure 2C). These results indicated that, within our mathematical model, low-normal FV levels and high-normal prothrombin levels enhanced thrombin generation in FVIII-deficient plasma and thus FV and FII became leading candidates as potential modifiers of thrombin generation.

Our mathematically identified candidates, FV and prothrombin, were then confirmed as modifiers by calibrated automated thrombography and microfluidic flow assays[59] on collagen-tissue factor surfaces at a shear rate of 100 s^{-1} utilizing both FVIII-inhibited plasma and whole blood from FVIII-deficient individuals. Using mixtures of FV and prothrombin-depleted plasmas, purified FV and prothrombin, and an anti-FVIII function–blocking antibody yielding FVIII activity of <1%, we generated four cases of variations in FII and FV plasma levels. Results were consistent with the mathematical model, showing that low-normal FV (43%) increased the peak thrombin concentration and endogenous thrombin potential and that both of these measures were further enhanced with high prothrombin (136%). Similar trends were seen with FVIII-deficient plasma from individuals using the same treatments as in the microfluidic flow assay experiments, see Figure 2D. Whole blood microfluidic assays were performed using both synthetic plasma and blood from individuals with moderate and mild FVIII deficiencies. There was a significant increase in both the rate and maximum accumulation of fibrin(ogen) with partial inhibition of FV via the anti-FV antibody, and an even greater response occurred with the addition of prothrombin, see Figure 2E.

What could be the basis of this enhanced response? Because our model is mechanistic, we are able to probe it further to determine which reactions in the coagulation system become more effective on the path to thrombin generation when FV is in the low-normal range. Doing so allowed us to recognize that in the model the mechanism for enhanced thrombin production is decreased substrate competition of platelet-bound FV with platelet-bound FVIII to bind to and be activated by platelet-bound FXa (activated factor X). In our simulations, FXa is the dominant activator of FV and FVIII before significant amounts of thrombin have been produced; therefore, FV and FVIII compete to form their respective substrate-enzyme complexes before their cleavage to FVa (activated factor V) and FVIIIa (activated factor FVIII). Model results showed higher concentrations of substrate-enzyme complexes (FVIII with FXa) for low-normal FV cases, indicating that lowering FV levels increases activation of FVIII by FXa. Further evidence in support of this mechanism is that decreasing the kinetic rate constant for the association of platelet-bound FV and FXa by 50% led to 30-fold higher thrombin concentrations (with normal FV levels) by 40 minutes. These results support the notion that reducing substrate competition between FV and FVIII for FXa is the mechanism (in the model) by which thrombin generation is rescued in FVIII-deficient plasma.

The model results and explorations of mechanism described above were performed under the assumption of severe hemophilia whereby extremely low levels of FVIII were used in the model (1% of normal or about 0.01 nmol/L) and for normal and low-normal levels of FV. Additional simulations showed that a further decrease in FV levels (down to about 10%) enhanced thrombin generation and further increase in FV levels (up to 200%) inhibits thrombin generation. Below an FV level of 10%, there was insufficient FV and FVIII to support significant thrombin generation (see Figure S2 in previous article[1]). Additionally, we observed variations in thrombin generation when decreasing FV levels from normal to low-normal levels for varying categories of hemophilia A, by using FVIII levels at 1, 3, 5, and 8% (see Figure S6 in our previous article[1]). For example, with FVIII at 8%, there was significant thrombin formed by 40 minutes when FV was at normal levels. But when FV was decreased to low-normal levels, there was enhanced thrombin generation as shown by a significant decrease in the time it took the system to achieve that thrombin concentration. These results are in line with a recent study by Shao et al[60] where peak thrombin concentration in calibrated automated thrombography was decreased when FV was titrated back into blood from individuals with combined FV/FVIII deficiencies.

CONCLUSIONS

In conclusion, computational modeling enables the creation of enormous synthetic patient data sets. These data sets are complementary to clinical or experimentally derived data sets in that they can be screened for specific metrics and statistically analyzed to direct small numbers of difficult experiments or identify biomarkers. As evidenced by our work described in this article, combining computationally driven and experimental approaches enhances the potential to discover modifiers

of coagulation. Further exploration with mechanistic dynamic models can provide focused analysis and predictions of any underlying biochemical mechanisms that lead to patterns in the synthetic data sets. Certainly, computational approaches have their limitations; the models should be simple enough to be truly computationally efficient to run but complex enough to include the relevant biochemistry and biophysics. That said, we think that computational approaches can help steer experimental approaches in directions that otherwise may not have been considered; even if a computational model fails to include one's favorite protein or reaction, they may still provide novel intuition about other proteins and interactions in coagulation, and can also usually be easily modified to consider more species or reactions. With advancements in computational power and technology, we think this is an exciting time to explore computational techniques for further discovery in coagulation.

ARTICLE INFORMATION

Received August 14, 2020; accepted September 30, 2020.

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Sources of Funding

This work was supported in part by the National Institutes of Health (R01HL120728, R01HL151984), the National Science Foundation (CBET-1351672, DMS-1848221), and the Army Research Office (ARO-12369656).

Disclosures

None.

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