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FLOW SIMULATED THROMBIN GENERATION PROFILES AS A PREDICTOR OF THROMBOTIC RISK AMONG PRE-MENOPAUSAL WOMEN

S. W. Jordan^{*}, M. A. Corriere^{†,‡}, C. Y. Vossen^{§,¶}, F. R. Rosendaal[§], and E. L. Chaikof^{*,**,††}

^{*}Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, USA [†]Division of Vascular Surgery, Emory University, Atlanta, GA, USA

[‡]Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA, USA

[§]Departments of Clinical Epidemiology and Thrombosis and Haemostasis, Leiden University Medical Center, Leiden, Netherlands [¶]Division of Biomedical Genetics, Department of Medical Genetics at the University Medical Center Utrecht, Utrecht, Netherlands

^{**}Department of Surgery, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA ^{††}Wyss Institute of Biologically Inspired Engineering of Harvard University, Boston, MA, USA

Summary

Background—A large number of individuals are at risk for deep venous thrombosis (DVT) due to alterations in multiple coagulation factors and inhibitors secondary to malignancy, drug interactions, or other general medical conditions. Traditional metrics of haemostasis such as prothrombin time, partial thromboplastin time, and bleeding time, generally estimate anticoagulation status and bleeding risk rather than thrombosis risk.

Objective—The objective of this study was to correlate a novel, systems-based metric of clotting potential to risk of DVT from a database derived from the Leiden Thrombophilia Study (LETS).

Methods—We utilized a computational model of blood coagulation, which addresses the interplay between biochemical factors, blood flow, and physiologic surface initiation of coagulation, to calculate an individualized, systems-based metric of clotting potential, termed the flow simulated thrombin generation (FSTG), for 210 pre-menopausal women in LETS.

Results—Both DVT and oral contraceptive (OC) use were associated with higher values of FSTG. We demonstrated a nearly 3-fold increased risk of DVT for each standard deviation increase above the mean in FSTG determined under venous flow conditions, which remained highly predictive after adjustment for age and OC status (adjusted OR 2.66; 95% CI 1.69–4.19; $P < 0.0001$).

Conclusions—A systems-based screening approach that integrates biochemical factors and flow haemodynamics identifies small subgroups of patients at risk of thrombosis that may benefit from oral anticoagulants.

Keywords

thrombosis; blood coagulation; computer simulation; thromboembolism

Contributors. SWJ and ELC conceived and designed the study, analyzed and interpreted the data, and wrote the first draft of the report. MAC analyzed and interpreted the data. CYV and FRR collected the data. All authors revised and reviewed the report.

Conflicts of interest: None.

As early as the 19th century, certain clinical settings have been associated with increased risk of thrombosis. These acquired thrombophilias include both rare and common conditions such as cancer, hormone therapy, nephrotic syndrome, antiphospholipid syndrome, and hyperhomocysteinemia. Oral contraceptive use is associated with a 2 to 6-fold increased risk of venous thrombosis, 6-fold increased risk of cerebral vein thrombosis [1], 5-fold increased risk of myocardial infarction, and 3-fold increased risk of ischemic stroke [2]. Several population-based studies have reported elevations in plasma levels of factors II, VII, VIII, X, and fibrinogen, lower plasma levels of factor V, antithrombin (AT), protein S (PS), and protein C (PS), and decreased activated partial thromboplastin time (aPTT).[3–6] Additionally, acquired APC resistance comparable to that found in heterozygous factor V Leiden has been observed in women prescribed third-generation oral contraceptives.[7, 8]

Presently, thrombophilia screening is limited and include protein C/S deficiencies, antithrombin deficiency, factor V Leiden polymorphism (G1691A), or APC resistance, prothrombin G20210A, dysfibrinogenemia, antiphospholipid antibody, lupus anticoagulant, and anticardiolipin antibody assays.[9] Other tools, such as the Caprini risk assessment model for peri-operative VTE risk are based on patient and operative risk factors such as age, past medical history, family history, oral contraceptive use, and surgical procedure.[10]

The Leiden Thrombophilia Study (LETS) has compiled extensive data regarding thrombotic risk associated with increases in individual plasma coagulation factor levels. Specifically, factors II, VIII, and IX had adjusted odds ratios calculated using a 90th percentile cutpoint of 2.1, 4.8, and 2.5, respectively, for first-time deep venous thrombosis (DVT).[11–13] However, it is generally accepted that changes in individual coagulation factors are not sufficiently predictive for assessing risk in a clinical setting due to the highly dynamic and complex nature of blood coagulation.[14] A systems-based approach, which does not rely on any one intermediary of coagulation, is required to adequately assess haemostatic status. In this study, we utilized a finite element model of surface-induced blood coagulation under flow to simulate thrombin generation based on patient coagulation factor profiles obtained from LETS and evaluated the association between simulated thrombin levels and risk of DVT among oral contraceptive users.

Materials and Methods

Study populations

LETS is a case-control study from the Netherlands with enrollment between 1988 and 1992 comparing participants with a first-time, objectively diagnosed DVT to sex and age-matched controls (N=474 per study arm).[11–13] Original exclusion criteria included known malignancies and age greater than 70 years. Blood samples were drawn between 6 and 56 months from the date of thrombosis. We analyzed pre-menopausal women whose OC status was known and unchanged at the time of thrombosis and blood draw. Additionally, participants on oral anticoagulants were excluded. Our study groups were composed of 80 case individuals and 130 control individuals. Coagulation factor levels were reported as percentages and were converted to molar concentrations by multiplying by published physiologic values (Table 1).[14–16]

Numerical model of thrombin generation

A finite element model (FEM) has been formulated that describes thrombin generation under defined flow conditions, initiated and modulated by spatially discrete regions of surface-bound tissue factor and thrombomodulin, respectively (Fig. 1).[17] The model incorporates fluid phase and surface-localized reactions of the extrinsic, intrinsic, and common pathways, as well as three major inhibitory pathways. The spatially heterogeneous

model describes coagulation events that occur following tissue factor exposure at a discrete site and incorporates mechanisms that regulate thrombin generation, including those mediated by an intact endothelium, represented as a thrombomodulin containing surface. Specifically, we assume that the coagulation cascade is initiated by binding of factor VIIa to tissue factor, leading to the formation of extrinsic tenase. Activation of factor X leads to activation of factors V and VII. The resultant surface-associated complex, Xa/Va, or prothrombinase, cleaves prothrombin to thrombin, which serves as a positive feedback loop by activating factors V and VIII. Generation of activated factor VIIIa, in association with IXa, affords intrinsic tenase, or IXa/VIIIa. We assume that factor IXa is generated solely by TF/VIIa without participation of intrinsic pathway factors XI and XII. Inhibitory reactions include the inactivation of factors IXa, Xa, and thrombin, by antithrombin. Additionally, inhibition of TF/VIIa by tissue factor pathway inhibitor (TFPI) is incorporated into the model as a two-step process, whereby TFPI binds factor Xa, followed by its reversible association with TF/VIIa. The protein C pathway serves as an additional negative feedback loop. Binding of thrombin to thrombomodulin leads to formation of activated protein C, which inactivates factors Va and VIIIa. Protein S directly modulates the kinetic efficiency of the latter inactivation reactions, but its interactions as a cofactor are not explicitly described.

The reaction area of interest is modeled as a two-dimensional flow field with upper and lower inert and reactive surfaces, respectively. Steady, one-dimensional, parabolic flow is assumed. Convective-diffusive transport is described by the conservation equation. All fluid-phase zymogen and enzyme concentrations are initially set to zero. The surface density of tissue factor and thrombomodulin remain constant, and at the outset both are free of bound cofactors. Convective flux transports 16 fluid phase species into the flow field at constant inlet concentrations derived from mean physiologic values, unless otherwise specified. Diffusion transports coagulation factors to the lower reactive surface, where reaction kinetics define flux boundary conditions. The reactive boundary is divided into localized upstream TF and downstream TM regions. The primary analysis was performed assuming a 10 mm entrance region, 2 mm TF region, and a 34 mm TM region. The length of the TF region is similar in dimension to that of a ruptured coronary plaque or vascular graft anastomosis.[18] The FEM model was implemented in COMSOL Multiphysics 3.4 (COMSOL, Inc., Burlington, MA) on a high performance computing (HPC) cluster (School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA). The automatically generated mesh contained approximately 2100 triangular elements, with finer resolution over the reactive boundaries and intersection points. Increasing mesh resolution to 3500 and 7200 triangular elements yielded results within 99% of the presented results. Soluble species were defined by a convection-diffusion application mode. Surface-bound enzymatic complex formation was specified by a weak form (boundary) application mode. Time dependent simulations were solved using a direct parallel solver. Simulations were run to 60 min. Post-processing was performed in both COMSOL and MATLAB (MathWorks, Inc., Natick, MA). All assumptions and implementation details along with basic validation of the model have been described elsewhere.[17]

An integrative parameter, flow simulated thrombin generation (FSTG), is derived from a consideration of the total thrombin generated beyond the site of TF expression at $y=0$ during the course of a 60-min flow period. One-hour was chosen as the default simulation time to ensure that all phases of coagulation, *e.g.* initiation and propagation, were included in the analysis for all runs. In other words, the simulation time exceeded the time course of the least thrombogenic simulation conditions. FSTG represents the average thrombin concentration over the surface of interest during the course of the simulation and acts as a global variable incorporating both spatial and temporal information. As described elsewhere, FSTG is a bimodal variable with values greater than 10 nM considered to exceed an activation threshold for a prothrombotic response. A value below 10 nM, notably the

minimum thrombin concentration reported to induce platelet activation,[19] was categorized as lying either below an activation-threshold. In order to obtain the greatest sensitivity for evaluating the effects of perturbations in haemostatic concentration profiles, conditions of the vessel wall, which normally result in flow simulated thrombin generation concentrations just below the activation threshold ($\text{FSTG} < 10 \text{ nM}$) under venous (75 s^{-1}) and arterial flow conditions (500 s^{-1}) were utilized for all simulations. These sub-threshold settings represent a 'pivot point' between a dampened thrombin response and full activation of the coagulation cascade and have been previously determined and include a tissue factor bearing injury zone that is 2 mm in length in which the density of TF is 0.07 and 0.8 fmol/cm^2 for venous and arterial conditions, respectively, and a downstream region presenting surface bound thrombomodulin at a concentration of 1000 fmol/cm^2 . While a different choice of parameters would result in quantitatively different thrombin responses, the comparative effects would be similar.

Statistical analysis

Female study participants whose OC status was known at the time of phlebotomy ($N=210$) were analyzed. Descriptive statistics are reported as mean $\pm$ standard deviation for continuous variables and number (%) for categorical variables. Group-wise comparisons were performed using T-tests or nonparametric methods for continuous variables (based on data distributions) and χ^2 tests for categorical variables.

FSTG was assessed as a continuous covariate in patients exceeding the 10 nM threshold for thrombin generation. Associations between venous ($\text{FSTG}_{\text{venous}}$) and arterial ($\text{FSTG}_{\text{arterial}}$) FSTG, coagulation factor levels, and DVT were evaluated using Receiver Operating Curve (ROC) analysis and logistic regression. Multivariable logistic regression modeling was performed using stepwise selection with $P = 0.10$ required for model entry and $P = 0.05$ required for covariate retention in the final model. DVT models based on coagulation factors or FSTG as covariates were adjusted post-hoc for participant age and oral contraceptive use. For continuous covariates, odds ratios (OR) are expressed per standard deviation change. Significance was assessed at an α level of 0.05 and all statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC).

Results

Descriptive statistics

Among pre-menopausal female participants in LETS ($N=210$), approximately 75% of the total population exceeded the 10 nM FSTG activation threshold ($N=159$ and $N=152$ for venous and arterial FSTG, respectively), with similar rates observed between control and DVT populations (Table 2). The remainder of individuals were predicted to have the lowest coagulable risk based on plasma factor levels alone. OC use had no effect on threshold rates within the DVT group, but increased the proportion of participants that exceeded the activation threshold within the control group.

Mean participant age was 38.2 ± 9.4 years. Mean venous and arterial FSTG were 435.0 ± 221.2 and $656.3 \pm 307.5 \text{ nM}$, respectively. Descriptive statistics stratified by DVT status are shown in Table 3. Higher concentrations of factors VII, VIII, IX, and X, and protein S were observed in the DVT population. No difference was noted for the remaining plasma concentrations. Stratified analysis based on DVT status revealed higher mean venous and arterial FSTG in the DVT group ($\text{FSTG}_{\text{venous}}$ 535.8 nM versus 372.3 nM, $P<0.0001$; $\text{FSTG}_{\text{arterial}}$ 789.8 nM versus 571.5 nM, $P<0.0001$) (Fig. 2). Stratified analysis based on OC use revealed higher venous and arterial mean FSTG in OC users ($\text{FSTG}_{\text{venous}}$ 514.5 nM versus 375.6 nM, $P<0.0001$; $\text{FSTG}_{\text{arterial}}$ 754.4 nM versus 576.8 nM, $P=0.0003$) (Fig. 3).

Patients using OC were also younger (mean age 32.5 ± 10.1 versus 41.8 ± 6.9 years; $P < 0.0001$).

Associations between FSTG and thrombosis

DVT occurred in 80 participants (38.1%). Univariate models of DVT are shown in Table 4. Venous and arterial FSTG demonstrated highly significant univariate associations with DVT [OR 2.26, 95% CI (1.56–3.30) for FSTG_{venous}; OR 2.23, 95% CI (1.51–3.30) for FSTG_{arterial}]. FSTG_{venous} and FSTG_{arterial} displayed comparable associations with DVT in ROC analyses (AUC 0.723 vs. 0.703, respectively; $P = 0.084$). FSTG_{venous} compared favorably with factors II, V, VII, TFPI, and protein C in univariate models of DVT ($P < 0.05$ for pair-wise comparisons), while curves were similar in pair-wise comparisons with factors VIII, IX, X, and protein S ($P > 0.05$ for pair-wise comparisons) (Fig. 4).

In multivariable modeling, associations with DVT were observed for FSTG_{venous} (OR 2.46; 95% CI (1.60–3.77); $P < 0.0001$) and protein S (OR 1.48; 95% CI (1.03–2.14); $P = 0.0332$) (Table 5). After adjusting the selected multivariable model for participant age and OC use, the association between DVT and FSTG_{venous} remained highly significant (OR 2.66; 95% CI 1.69–4.19; $P < 0.0001$), but the adjusted OR for protein S was no longer significant (OR 1.24; 95% CI (0.80–1.91); $P = 0.3318$).

Discussion

Patient screening and clinical assessment

Given the large population at risk for thrombophilia, whether genetic or acquired, and the complexities of integrating multiple risk factors, *e.g.* a FV Leiden carrier wishing to take oral contraceptives, there exists a need for a diagnostic tool to assess an individual's haemostatic state. Due to low absolute risk, the cost-effectiveness of universal screening has been disparaged.[20, 21] However, selective thrombophilia screening and subsequent optimization of prophylactic treatment may improve overall clinical outcomes.[20, 22] The primary methods of haemostatic assessment remain 'prothrombin time' (PT) and 'activated partial thromboplastin time' (aPTT), both relatively insensitive to thrombotic risk. A more global approach, the thrombin generation assay (TGA) uses a fluorogenic-based method to measure thrombin concentration over time in platelet-poor plasma, platelet-rich plasma, or whole blood. In 1993, Hemker *et al.*[23] defined the term 'endogenous thrombin potential' (ETP) as the area under the curve of the thrombin generation curve, or 'thrombogram', and proposed the ETP as a sensitive marker of the capacity of plasma to form thrombin in response to a stimulus. A number of studies have been performed to evaluate the predictive value of ETP with respect to hyper- and hypocoagulable states and haemostatic risk.[24] However, there remains significant inter-laboratory variability, and large prospective clinical trials are lacking.

Computational methods in conjunction with individual coagulation protein profiles have the potential to be high throughput screening tools given recent advances in both biochemical assays and computer processing. Brummel-Ziedins *et al.*[25] input coagulation factor data from the control population of LETS into a 27-equation computational model and examined the variation in simulated thrombin generation curves stratified by risk group, such as sex, increased body mass index ($\text{BMI} > 26 \text{ kg/m}^2$), and use of oral contraceptives.[26] Input parameters included factors II, V, VII, VIII, IX, X, and the inhibitors ATIII and TFPI. Output parameters included time to 10 nM of thrombin, time to maximum thrombin, maximum rate of thrombin generation (MaxR), maximum level of thrombin generation (MaxL), and total thrombin generated. Together these parameters described the initiation, propagation, and termination phases of thrombin generation triggered by a fixed quantity of

TF in solution. Interestingly, their study identified subpopulations, termed ‘fast- and slow-thrombin generators’, which the authors proposed represented categories of risk. OC use was associated with increases in thrombin-related parameters in both control and DVT populations. The reported OR using the 90th percentile cutpoint for their greatest risk-associated parameter, MaxR, and OC use was 2.9 (95% CI 1.1–7.7). Brummel-Ziedens *et al.* proposed the combination of an individual’s plasma coagulation profile with a computational tool for evaluation of thrombotic risk, as well as assessment of global haemostatic changes following the initiation of oral contraceptives. The present study further includes explicit incorporation of the protein C pathway and a well defined flow regime.

Assumptions and limitations

Given the known association of OC use with thrombosis, we chose to focus on a cohort of pre-menopausal females with known and consistent OC status at the time of thrombosis and blood draw. This subset of the original LETS case-control data set, matched by age and sex, may not be fully representative of the entire population. However, FSTG_{venous} remained highly associated with DVT when adjusted for demographic variables such as age and OC use, while other coagulation variables did not. Further studies of FSTG_{venous} adjusting for other known risk factors of DVT including smoking, obesity, and ethnicity are warranted to determine a clinically relevant cutpoint for FSTG_{venous}.

It bears emphasis that the reaction pathways and related kinetic parameters are largely well-defined for the coagulation cascade, and furthermore, that no parameter fitting was performed for our model system to calculate FSTG. Kinetic parameters were critically selected from the experimental literature of isolated reaction systems. Given a different set of physiologically relevant parameters, the qualitative trends observed in our studies would have remained the same, and thus the model would still be useful for the comparison of altered haemostatic parameters.

While the inclusion of surface-bound TF and TM renders the computational model more physiologically relevant, diagnostically, there are no direct measures of alterations in TF or TM surface expression. Circulating TF on the surface of monocytes and microparticles may be detected by flow cytometry, but their role in coagulation and the correlation to endothelial and subendothelial surface expression remain undefined at this time.[27–30] In contrast, soluble thrombomodulin has been established as a marker of early endothelial cell damage.[31, 32] Kunz *et al.* utilized monocyte expression of TM as an *in vivo* marker of endothelial cell expression in a familial study of TM deficiency. However, the ability to quantify the extent of reduced TM expression in endothelial cells was limited by the relatively low levels of TM found on the surface of circulating monocytes. [33] As measurement methods continue to evolve, TF and TM concentrations could easily be incorporated into our existing computational model. Additionally mechanistic inclusion of protein S or the incorporation of laboratory APC sensitivity measurements may be useful for the representation and study of pathologic states.

A systems-based method to predict thrombosis

Our univariate analysis recapitulated some of the findings previously published by the LETS group regarding the association of elevations in certain prothrombotic factors with venous thrombosis.[11–13] Adjusted for demographic variables, age and OC use, the systems-based variables, *i.e.* FSTG_{venous} and FSTG_{arterial}, had the highest predictive value, followed by the individual factors VIII, IX, and X. When all candidate covariates were allowed to compete and adjusted for age and OC use, only FSTG_{venous} remained significant. Notably,

FSTG_{venous} is a robust risk-associated variable with little perturbation in the adjusted OR observed, regardless of logistic model selection.

We have utilized a computational model to calculate a systems-based, mechanism-based, metric of clotting potential, FSTG_{venous}. We demonstrated a 166% increased risk of DVT for each SD (221 nM) increase in FSTG_{venous} above the mean (435 nM) for our study population. While no one plasma marker is sufficiently predictive of thrombotic risk, a systems-based screening approach is both logical and necessary to identify high-risk patients. For this small subgroup of patients, the bleeding and pharmacologic risks associated with prophylactic anticoagulation are outweighed by the risk of long-term morbidity from DVT and the risk of mortality from PE. Objective measurements will improve both cost-effectiveness as well as individual patient safety and health.

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References

1. Dentali F, Crowther M, Ageno W. Thrombophilic abnormalities, oral contraceptives, and risk of cerebral vein thrombosis: A meta-analysis. *Blood*. 2006; 107(7):2766–73. [PubMed: 16397131]
2. Martinelli, I., editor. Risk factors in venous thromboembolism; XVIIIth Congress of the International-Society-on-Thrombosis-and-Haemostasis; 2001 Jul 06–12; Paris, France: F K Schattauer Verlag GmbH;
3. Bloemenkamp KWM, Helmerhorst FM, Rosendaal FR, Vandenbroucke JP. Venous thrombosis, oral contraceptives and high factor viii levels. *Thrombosis and Haemostasis*. 1999; 82(3):1024–7. [PubMed: 10494758]
4. Mackie IJ, Piegsa K, Furs SA, Johnson J, Bounds W, Machin SJ, et al. Protein s levels are lower in women receiving desogestrel-containing combined oral contraceptives (cocs) than in women receiving levonorgestrel-containing cocs at steady state and on cross-over. *British Journal of Haematology*. 2001; 113(4):898–904. [PubMed: 11442481]
5. Quehenberger P, Loner U, Kapiotis S, Handler S, Schneider B, Huber J, et al. Increased levels of activated factor vii and decreased plasma protein s activity and circulating thrombomodulin during use of oral contraceptives. *Thrombosis and Haemostasis*. 1996; 76(5):729–34. [PubMed: 8950781]
6. Tans G, Curvers J, Middeldorp S, Thomassen M, Meijers JCM, Prins MH, et al. A randomized cross-over study on the effects of levonorgestrel- and desogestrel-containing oral contraceptives on the anticoagulant pathways. *Thrombosis and Haemostasis*. 2000; 84(1):15–21. [PubMed: 10928463]
7. Rosing J, Middeldorp S, Curvers J, Thomassen M, Nicolaes GAF, Meijers JCM, et al. Low-dose oral contraceptives and acquired resistance to activated protein c: A randomised cross-over study. *Lancet*. 1999; 354(9195):2036–40. [PubMed: 10636369]
8. Rosing J, Tans G, Nicolaes GAF, Thomassen M, vanOerle R, vanderPloeg P, et al. Oral contraceptives and venous thrombosis: Different sensitivities to activated protein c in women using second- and third-generation oral contraceptives. *British Journal of Haematology*. 1997; 97(1):233–8. [PubMed: 9136971]
9. Favaloro EJ, McDonald D, Lippi G. Laboratory investigation of thrombophilia: The good, the bad, and the ugly. *Semin Thromb Hemost*. 2009; 35(7):695–710. Epub 2009/12/17. [PubMed: 20013536]
10. Pannucci CJ, Bailey SH, Dreszer G, Fisher Wachtman C, Zumsteg JW, Jaber RM, et al. Validation of the caprini risk assessment model in plastic and reconstructive surgery patients. *Journal of the American College of Surgeons*. 2011; 212(1):105–12. Epub 2010/11/26. [PubMed: 21093314]
11. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood*. 1996; 88(10):3698–703. [PubMed: 8916933]

12. Koster T, Blann AD, Briet E, Vandenbroucke JP, Rosendaal FR. Role of clotting factor-viii in effect of von-willebrand-factor on occurrence of deep-vein thrombosis. *Lancet*. 1995; 345(8943): 152–5. [PubMed: 7823669]
13. Vlieg AV, van der Linden IK, Bertina RM, Rosendaal FR. High levels of factor ix increase the risk of venous thrombosis. *Blood*. 2000; 95(12):3678–82. [PubMed: 10845896]
14. Butenas S, van't Veer C, Mann KG. "Normal" thrombin generation. *Blood*. 1999; 94(7):2169–78. [PubMed: 10498586]
15. Murano G, Williams L, Miller-Andersson M, Aronson DL, King C. Some properties of antithrombin-iii and its concentration in human plasma. *Thromb Res*. 1980; 18(1–2):259–62. Epub 1980/04/01. [PubMed: 6773181]
16. Novotny WF, Brown SG, Miletich JP, Rader DJ, Broze GJ Jr. Plasma antigen levels of the lipoprotein-associated coagulation inhibitor in patient samples. *Blood*. 1991; 78(2):387–93. Epub 1991/07/15. [PubMed: 2070076]
17. Jordan SW, Chaikof EL. Simulated surface-induced thrombin generation in a flow field. *Biophys J*. 2011; 101(2):276–86. Epub 2011/07/20. [PubMed: 21767479]
18. Nakazawa G, Finn AV, Joner M, Ladich E, Kutys R, Mont EK, et al. Delayed arterial healing and increased late stent thrombosis at culprit sites after drug-eluting stent placement for acute myocardial infarction patients - an autopsy study. *Circulation*. 2008; 118(11):1138–45. [PubMed: 18725485]
19. Brummel KE, Paradis SG, Butenas S, Mann KG. Thrombin functions during tissue factor-induced blood coagulation. *Blood*. 2002; 100(1):148–52. [PubMed: 12070020]
20. Martinelli I. Pros and cons of thrombophilia testing: Pros. *Journal of Thrombosis and Haemostasis*. 2003; 1(3):410–1. [PubMed: 12871438]
21. Vandenbroucke JP, vanderMeer FJM, Helmerhorst FM, Rosendaal FR. Factor v leiden: Should we screen oral contraceptive users and pregnant women? *British Medical Journal*. 1996; 313(7065): 1127–30. [PubMed: 8916702]
22. Wu O, Robertson L, Twaddle S, Lowe G, Clark P, Walker I, et al. Screening for thrombophilia in high-risk situations: A meta-analysis and cost-effectiveness analysis. *British Journal of Haematology*. 2005; 131(1):80–90. [PubMed: 16173967]
23. Hemker HC, Wielders S, Kessels H, Beguin S. Continuous registration of thrombin generation in plasma, its use for the determination of the thrombin potential. *Thrombosis and Haemostasis*. 1993; 70(4):617–24. [PubMed: 7509511]
24. van Veen JJ, Gatt A, Makris M. Thrombin generation testing in routine clinical practice: Are we there yet? *British Journal of Haematology*. 2008; 142(6):889–903. [PubMed: 18564356]
25. Brummel-Ziedins K, Vossen CY, Rosendaal FR, Umezaki K, Mann KG. The plasma hemostatic proteome: Thrombin generation in healthy individuals. *Journal of Thrombosis and Haemostasis*. 2005; 3(7):1472–81. [PubMed: 15978105]
26. Hockin MF, Jones KC, Everse SJ, Mann KG. A model for the stoichiometric regulation of blood coagulation. *Journal of Biological Chemistry*. 2002; 277(21):18322–33. [PubMed: 11893748]
27. Kushak RI, Nestoridi E, Lambert J, Selig MK, Ingelfinger JR, Grabowski EF. Detached endothelial cells and microparticles as sources of tissue factor activity. *Thrombosis Research*. 2005; 116(5):409–19. [PubMed: 16122554]
28. Mallat Z, Hugel B, Ohan J, Leseche G, Freyssinet JM, Tedgui A. Shed membrane microparticles with procoagulant potential in human atherosclerotic plaques - a role for apoptosis in plaque thrombogenicity. *Circulation*. 1999; 99(3):348–53. [PubMed: 9918520]
29. Nieuwland R, Berckmans RJ, RotteveelEijkman RC, Maquelin KN, Roozendaal KJ, Jansen PGM, et al. Cell-derived microparticles generated in patients during cardiopulmonary bypass are highly procoagulant. *Circulation*. 1997; 96(10):3534–41. [PubMed: 9396452]
30. Satta N, Toti F, Feugeas O, Bohbot A, Dacharyprigent J, Eschwege V, et al. Monocyte vesiculation is a possible mechanism for dissemination of membrane-associated procoagulant activities and adhesion molecules after stimulation by lipopolysaccharide. *Journal of Immunology*. 1994; 153(7): 3245–55.

31. Boehme MWJ, Galle P, Stremmel W. Kinetics of thrombomodulin release and endothelial cell injury by neutrophil-derived proteases and oxygen radicals. *Immunology*. 2002; 107(3):340–9. [PubMed: 12423310]
32. Ishii H, Uchiyama H, Kazama M. Soluble thrombomodulin antigen in conditioned medium is increased by damage of endothelial-cells. *Thrombosis and Haemostasis*. 1991; 65(5):618–23. [PubMed: 1651569]
33. Kunz G, Ireland HA, Stubbs PJ, Kahan M, Coulton GC, Lane DA. Identification and characterization of a thrombomodulin gene mutation coding for an elongated protein with reduced expression in a kindred with myocardial infarction. *Blood*. 2000; 95(2):569–76. [PubMed: 10627464]

What is known about thrombotic risk

- Current clinical risk models are based on population-based studies of patient demographics and comorbidities.
- Several genetic and environmental factors contribute to a patient's risk of VTE, partly due to alterations in the patient's individual coagulation factor profile.

What this paper contributes

- Simulated thrombin generation is a better predictor of thrombosis risk than any single biochemical marker.
- Computational modeling may be a useful tool in assessing a patient's thrombotic risk based on their individual biochemical profile.

Figure 1. Formulation of a spatially heterogeneous model of surface-induced thrombin generation in a flow field

(A) Blood flow carries proteins into a flow field simulated as simple, laminar, parabolic flow with velocity, u and wall shear rate, $\dot{\gamma}$. Conservation equations describe diffusion of coagulation factors (diffusivity, D) to the lower reactive surface ($y=0$), where flux boundary conditions define reaction kinetics. Convective flux removes soluble species at the outlet. B is the half-height of the chamber, R describes solution phase reaction kinetics. (B) Geometric conditions include a 10 mm inert entrance region (gray), 2 mm thrombin-initiating TF region (pink), and a 34 mm anti-thrombogenic TM region (blue). The upper boundary is inert. Flow simulated thrombin generation (FSTG) is defined as the integral of the downstream thrombin surface concentration over the 60-min simulation time, normalized by time and length. (C) A representative thrombin concentration profile at 60-min is presented.

Figure 2. Venous and arterial FSTG distribution stratified by DVT status

Box plots represent median, 10th, 25th, 75th, and 90th percentiles. Open circles denote 5th and 95th percentiles. Dashed lines indicate the mean. Asterisks indicate statistical significance. Flow simulated thrombin generation (FSTG) simulated over a 60-min flow period at shear rates of (A) 75 s⁻¹ and (B) 500 s⁻¹.

Figure 3. Venous and arterial FSTG distribution stratified by OC use

Box plots represent median, 10th, 25th, 75th, and 90th percentiles. Open circles denote 5th and 95th percentiles. Dashed lines indicate the mean. Asterisks indicate statistical significance. Flow simulated thrombin generation (FSTG) simulated over a 60-min flow period at shear rates of (A) 75 s⁻¹ and (B) 500 s⁻¹.

Figure 4. Receiver Operating Curve comparisons for candidate covariates

* $p < 0.05$ versus FSTG_{venous}

Table 1

Reference plasma concentrations

Coagulation factor	Plasma level (nM)	Ref.
Factor II	1400	[14]
Factor V	20	[14]
Factor VII	10	[14]
Factor VIII	0.7	[14]
Factor IX	90	[14]
Factor X	170	[14]
Antithrombin III	2400	[15]
Protein C	60	[14]
Protein S	300	[14]
TFPI	2.5	[16]

Table 2aNumber of participants with $\text{FSTG}_{\text{venous}} > 10 \text{ nM}$

Group	DVT	
	No	Yes
All participants	98/130 (75.3)	61/80 (76.3)
Non-OC users	53/80 (66.3)	38/50 (76.0)
OC users	45/50 (90.0)	23/30 (76.7)

Data is displayed as N/N_{total} (%)

Table 2bNumber of participants with $\text{FSTG}_{\text{arterial}} > 10 \text{ nM}$

Group	DVT	
	No	Yes
All participants	93/130 (71.5)	59/80 (73.8)
Non-OC users	49/80 (61.3)	35/50 (70.0)
OC users	44/50 (88.0)	24/30 (80.0)

Data is displayed as N/N_{total} (%).

Table 3

Descriptive statistics for the entire study population *

Variable	DVT	
	No	Yes
Age (years)	38.3 ± 9.2	38.0 ± 9.7
OCP use	50 (38.5)	30 (37.5)
Factor II (%)	103.8 ± 15.2	109.2 ± 17.9
Factor V (%)	119.6 ± 28.7	122.9 ± 32.2
Factor VII (%)	110.0 ± 22.3	109.3 ± 17.9
Factor VIII (%)	119.8 ± 31.4	135.4 ± 34.2
Factor IX (%)	102.4 ± 22.6	113.6 ± 30.2
Factor X (%)	104.8 ± 21.1	114.0 ± 23.0
Antithrombin III (%)	98.6 ± 10.5	97.2 ± 12.0
Protein C (%)	101.5 ± 16.5	107.1 ± 24.2
Protein S (%)	91.8 ± 18.5	99.0 ± 19.6
TFPI (ng/mL)	59.1 ± 13.9	59.7 ± 12.0
Venous FSTG (nM)	372 ± 196	536 ± 224
Arterial FSTG (nM)	572 ± 285	790 ± 296

* Data are displayed as mean ± standard deviation for continuous variables and N (%)

for categorical variables. Factor concentrations are reported as % of normal.

Table 4

Univariate models with DVT as an outcome

Variable	OR* (95% CI)	P
Venous FSTG	2.26 (1.56–3.30)	< 0.0001
Factor II	1.40 (1.05–1.87)	0.0227
Factor V	1.12 (0.85–1.45)	0.4381
Factor VII	1.39 (1.04–1.85)	0.0251
Factor VIII	6.37 (2.07–19.62)	0.0013
Factor IX	1.54 (1.15–2.05)	0.0032
Factor X	1.53 (1.14–2.05)	0.0043
ATIII	0.88 (0.66–1.16)	0.3597
Protein C	1.33 (0.99–1.76)	0.0510
Protein S	1.46 (1.10–1.95)	0.0097
TFPI	1.05 (0.80–1.38)	0.7409

* Odds ratios for expressed per standard deviation change.

Table 5

Multivariable models with DVT as outcome

Variable	Unadjusted		Adjusted*	
	OR (95% CI)	P	OR (95% CI)	P
Venous FSTG	2.46 (1.60–3.77)	< 0.0001	2.66 (1.69–4.19)	< 0.0001
Protein S	1.48 (1.03–2.14)	0.0332	1.24 (0.80–1.91)	0.3318

* Adjusted for participant age and oral contraceptive use at time of phlebotomy