


ORIGINAL ARTICLE

Anticoagulated patients exhibit intact endogenous thrombin potential using ST Genesis unlike the Calibrated Automated Thrombogram

Tuukka A. Helin MD, PhD¹  | Marja Lemponen BSC¹ | Riitta Lassila MD, PhD² | Lotta Joutsu-Korhonen MD, PhD¹

¹Department of Clinical Chemistry, HUS Diagnostic Center, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

²Coagulation Disorders Unit, Helsinki University Hospital, Research Program Unit in Systems Oncology, University of Helsinki, Helsinki, Finland

Correspondence

Lotta Joutsu-Korhonen, Department of Clinical Chemistry, HUS Diagnostic Center, POB 720, 00029 Helsinki, Finland.
Email: lotta.joutsu-korhonen@hus.fi

Funding information

Diagnostica Stago (France) allowed access to use the ST Genesis device for this study. The study did not receive any other funding.

Handling Editor: Pantep Angchaisuksiri.

Abstract

Background: The thrombin generation (TG) assay is a feasible but labor-intensive method for detecting global coagulation. It enables comprehensive assessment of anticoagulation, while drug-specific assays assess only exposure. Traditionally, the Calibrated Automated Thrombogram (CAT) has been used, however the ST Genesis (Diagnostica Stago) allows automated evaluation.

Objective: We aimed to observe coagulation using the ST Genesis and compare the data with those of CAT in anticoagulated patients.

Patients and methods: In total, 43 frozen-thawed samples were studied using DrugScreen to assess direct oral anticoagulants (DOACs), warfarin, and low-molecular-weight heparin. Twenty samples (nine rivaroxaban, five apixaban, three warfarin, and three heparin) were also compared using CAT (5 pM tissue factor).

Results: TG reduction in DrugScreen depended on the specific drug and modestly correlated with DOAC levels (lag time $R^2 = 0.36$; peak $R^2 = 0.50$). The best correlation was observed with peak thrombin and rivaroxaban-specified anti-activated factor X (anti-Xa) activity ($R^2 = 0.60$). When comparing ST Genesis with CAT, only the results for apixaban concurred ($R^2 = 0.97$). Unlike CAT, ST Genesis yielded a normal endogenous thrombin potential (ETP) in 77% (24/31) activated factor X inhibitor cases, and it failed to give readouts at international normalized ratio (INR) ≥ 4.5 and at anti-Xa ≥ 1.0 IU/mL.

Conclusion: The ST Genesis data did not correlate with CAT, but it was independently associated with INR, anti-Xa, and DOAC concentrations. The lag time and peak responses were similar; the major differences were that ST Genesis showed no ETP effect of DOACs and failed to give readout at high INR or anti-Xa activity.

KEYWORDS

anticoagulants, calibration, heparin, thrombin/analysis, warfarin

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2021 The Authors. *Research and Practice in Thrombosis and Haemostasis* published by Wiley Periodicals LLC on behalf of International Society on Thrombosis and Haemostasis (ISTH).

Essentials

- The new ST Genesis DrugScreen method has been suggested for monitoring anticoagulation.
- We compared ST Genesis with the established Calibrated Automated Thrombogram (CAT) method.
- DrugScreen showed different sensitivities to direct oral anticoagulants than CAT.
- DrugScreen failed to give a readout at high international normalized ratio or anti-Xa activity.

1 | INTRODUCTION

The thrombin generation (TG) assay is often used to assess coagulation in research settings. It can be used to monitor anticoagulant response, as well as to identify coagulation deficiencies and prothrombotic tendencies.¹ The semi-automated Calibrated Automated Thrombogram (CAT; Thrombinoscope, Diagnostica Stago, Asnières, France) method has been available to evaluate the TG for more than 2 decades.^{2,3} Although the CAT has proven useful in several clinical settings, such as when using hemophilia bypassing agents and emicizumab, the method is labor intensive and poorly standardized. For this reason, it has mainly been restricted to research use.^{4,5} The ex vivo capacity of citrated recalcified plasma to generate thrombin is measured by adding phospholipids and tissue factor (TF).

A new device, ST Genesis (Diagnostica Stago), enables automated TG measurement, which would make the assay more useful in clinical settings. However, the method is still being validated in patients, particularly those using anticoagulants. Currently, ST Genesis has three available test reagents: BleedScreen, ThromboScreen, and DrugScreen, which have low, intermediate, and high TF content, respectively. The exact TF concentrations are not publicly available. DrugScreen is recommended for measuring anticoagulant effects. The TF component is associated with the sensitivity of the assay to anticoagulants; the TG curve is negligible if TF is used at too low a concentration. Recently, the validation results and reference intervals of ST Genesis were reported in healthy volunteers.⁶

The ST Genesis and CAT assays have both practical and methodological differences (Table 1). Both are fluorogenic methods that use a substrate split by thrombin after initiation, with supplementation of varying amounts of TF and phospholipids into the plasma. In ST Genesis, TG is measured in single cuvettes that require a higher plasma sample volume, while microtiter plates are used in CAT. ST Genesis is calibrated using buffered human thrombin without patient plasma, unlike CAT, which uses alpha-2-macroglobulin. ST Genesis is calibrated daily. To this end, the individual plasma properties are compared using the FluoSet reagent. In CAT, each patient sample is run in parallel with an alpha-2-macroglobulin calibrant. Due to this difference, dabigatran likely has less influence on ST Genesis than on CAT.⁶⁻⁸

Few studies have compared CAT with ST Genesis.⁹ Recently, the plasma levels of direct oral anticoagulants (DOACs) were shown to correlate with the lag time and peak height of the DrugScreen test.⁷ However, no studies have compared the DrugScreen test

with the CAT assay in anticoagulated patient samples. In the present study, we aimed to verify the ST Genesis DrugScreen test in patients using different anticoagulants at different plasma concentrations. We also compared ST Genesis with the more established CAT method. DOACs, low-molecular-weight heparin (LMWH), and warfarin plasma samples were included in this comparison.

2 | MATERIALS AND METHODS

Blood samples were collected and assays performed at the Department of Clinical Chemistry of the Helsinki University Hospital (HUSLAB Laboratory Services, Finland). The samples comprised surplus plasma from clinical thrombophilia panels or DOAC concentration assessments. Citrated plasma (3.2% sodium citrate) was collected, centrifuged at 2500 g for 10 minutes, aliquoted within 2 hours of collection, and frozen at -80°C . All requested routine coagulation test analyses were freshly performed using ACL TOP coagulation analyzers (Instrumentation Laboratory, Werfen, Columbia, MD, USA), including international normalized ratio (INR), anti-activated factor Xa (anti-Xa), diluted thrombin time calibrated with dabigatran, and the anti-Xa method calibrated using either rivaroxaban or apixaban. For all the anti-Xa assays, we used the HemosIL Liquid anti-Xa (Instrumentation Laboratory, Werfen), which contains dextran sulfate (may affect heparin measurement) but no added exogenous antithrombin.¹⁰ The TG analyses were performed within 1-2 weeks of sample collection. No corn trypsin inhibitors were added. All samples were anonymously analyzed, and thus the specific indications for anticoagulation were not available. The study received institutional approval from Helsinki University Hospital (HUS/211/2020; § 34, 30.9.2020). We compared the response of the CAT 5 pM TF reagent (Thrombinoscope, Diagnostica Stago) with those of the ST Genesis ThromboScreen and DrugScreen reagents (Diagnostica Stago).

2.1 | DOAC plasmas

We collected 31 samples from patients using DOACs: 4 using dabigatran, 17 using rivaroxaban, and 10 using apixaban. Six patient samples were used in the pilot study: three using rivaroxaban, two using apixaban, and one using edoxaban.

TABLE 1 Characteristics of ST Genesis DrugScreen and CAT assays to measure thrombin generation

	ST Genesis DrugScreen	CAT with 5 pM TF
Detection principle	Fluorogenic	Fluorogenic
Calibration	Buffered human thrombin	Alpha-2-macroglobulin
Control frequency	Once daily	Each plate
TF concentration	High concentration ^a	5 pM
Parallel samples in one run	20-sample	22 samples (in duplicate)
Single sample testing possible	Yes, single cuvettes	Yes, but whole plate is used
Sample turnaround time	Preparation ~120 min, analysis 40–60 min	Preparation ~20 min, analysis 60 min
Automation	Fully automated	Plate analysis automatic, manual pipetting
Plasma volume per sample	800 µL	320 µL (measurement and calibration in duplicate, 80 µL each)

Abbreviations: CAT, Calibrated Automated Thrombogram; TF, tissue factor.

^aManufacturer does not specify exact concentration.

TABLE 2 Warfarin and heparin samples assessed with both ST Genesis DrugScreen and CAT at 5 pM TF

Anticoagulant sample	INR	Anti-Xa (IU/mL)	ST Genesis DrugScreen				CAT with 5 pM TF			
			Lag time (min)	Time to peak (min)	Peak Height (nM)	ETP (nM·min)	Lag time (min)	Time to peak (min)	Peak (nM)	ETP (nM·min)
Normal pool	1.0	NA	1.30	2.46	321.3	1169	3.33	6.67	172.2	1074
Warfarin pool	2.0	NA	2.49	3.96	147.6	568	6.67	10.33	78.6	479
Warfarin pool	2.5	NA	2.60	4.01	123.7	487	7.33	10.67	63.8	356
Warfarin pool	3.0	NA	2.86	4.35	101.5	396	8.50	12.17	49.3	321
Warfarin pool	4.5	NA	No result ^a	No result ^a	No result ^a	No result ^a	14.67	18.83	22.6	168
Warfarin pool	6.5	NA	No result ^a	No result ^a	No result ^a	No result ^a	23.00	30.33	6.8	71
Warfarin pool	>9.9	NA	No result ^a	No result ^a	No result ^a	No result ^a	NA ^b	NA ^b	NA ^b	NA ^b
Heparin pool	NA	<0.1	1.82	3.26	313.3	1267	5.83	9.5	148.0	1020
Heparin pool	NA	0.3	3.07	5.08	194.9	969	9.67	16.67	18.2	266
Heparin pool ^c	NA	0.5	3.92	6.69	89.0	578	20.67	32.5	3.1	55
Heparin sample	NA	1.0	No result ^a	No result ^a	No result ^a	No result ^a	NA ^b	NA ^b	NA ^b	NA ^b
Heparin sample	NA	1.2	No result ^a	No result ^a	No result ^a	No result ^a	NA ^b	NA ^b	NA ^b	NA ^b

Abbreviations: CAT, Calibrated Automated Thrombogram; ETP, endogenous thrombin potential; NA, not applicable; TF, tissue factor.

^aNo readout of thrombin generation (the curve was too flat).

^bCAT analysis not done due to limited sample volume.

^cGenesis DrugScreen and CAT results obtained from two different pools.

2.2 | Pooled warfarin and LMWH plasmas

We also prepared a series of pooled plasmas from samples in which treatment with warfarin or the LMWH effect was measured. There were five patient samples per pool, except in the high INR (>9.9) pool, where two samples were used. Different warfarin samples (n = 38) were pooled to obtain a series of plasma samples covering INR levels ranging from 1.0 to >9.9 (Table 2). Different LMWH samples (n = 31) were pooled to obtain plasma samples with wide anti-Xa levels, from <0.1 to 1.2 IU/mL (Table 2).

2.3 | ST Genesis DrugScreen anticoagulant response and comparison with CAT assay

The following TG parameters were recorded: lag time (minutes), time to peak (minutes), peak height (nM), and endogenous thrombin potential (ETP) (nM × min). The methods were compared with consideration for the different TF concentrations of the reagents. For comparison, the same pooled samples or patient samples (different frozen aliquots) were used in parallel to assay DrugScreen and CAT. The TG assays were analyzed without adding thrombomodulin.

2.4 | Statistical analysis

Both devices have specific software programs: ST Genesia uses a software of the same name, while CAT uses Thrombinoscope. Pearson correlations or Bland-Altman plots were used as appropriate. Statistical analyses were performed using SPSS Statistics version 25 (IBM, Armonk, NY, USA).

3 | RESULTS

3.1 | Pilot study for ST Genesia

A pilot was conducted using ST Genesia ThromboScreen to analyze six DOAC and five warfarin plasma samples. We assumed that the intermediate TF concentration would best represent the concentration of 5 pM in the CAT assay. Because the TG curves were flat and INR levels >4.5 could not be analyzed, we selected the DrugScreen with its higher TF content for the subsequent studies.

3.2 | ST Genesia DrugScreen in samples with anticoagulation

After the pilot, 43 samples were assessed in 31 individual DOAC samples, 7 INR pooled samples, and 5 LMWH pooled samples.

For reference, we analyzed the normal plasma pool sample ($n = 10$ healthy donors) 13 times in parallel. In this pooled analysis, the mean lag time was 1.19 minutes, the time to peak 2.34 minutes, the peak height 453 nM, and the ETP 1610 nM \times min, with the intra-assay coefficients of variation (CVs) between 1.9% and 2.8%. These values correspond well with those reported in the literature. The normal reference values for ST Genesia DrugScreen are: lag time, 0.82-1.00 minutes; peak, 380-539 nM; time to peak, 1.87-2.17 min; and ETP, 1122-1851 nM \times min⁶, with a good precision and stability (CV <3% for all variables). These reported reference values were used to interpret our own results.

The ST Genesia results correlated with increasing concentrations of each anticoagulant, that is, with increasing DOAC concentrations, INRs, and anti-Xa values (Figure 1, Table 2, Figure 2). The lag time and time to peak were prolonged in all anticoagulated samples (Figure 1A-B, Table 2). Peak height was lowered in all warfarin and LMWH samples and in most DOAC samples (Figure 1 and 2). The peak was normal in only three anti-Xa inhibitor samples in which drug concentrations were <100 ng/mL (Figure 1C, Table 2).

When the anticoagulation levels were within the expected treatment ranges, the peak values correlated best with the DOAC concentration in the rivaroxaban samples ($R^2 = 0.60$), while they correlated somewhat in the apixaban samples ($R^2 = 0.46$ after excluding the highest apixaban sample of 795 ng/mL as an outlier). Lag time and time to peak doubled when the DOAC concentrations increased from <100 ng/mL to >200 ng/mL (Figure 1A-B), while the peak halved from 200-300 nM to around 100 nM (Figure 1C). In the

warfarin and LMWH samples, lag time and time to peak were 1.5- to 2-fold higher at treatment levels than at baseline. The peak at an INR level of 3.0 was around 30% of that at an INR of 1.0 (Table 2). The ETP was also reduced in the warfarin and LMWH samples, being 50%-60% lower than normal (Table 2). In sharp contrast, the ETP remained within the reference interval in 24 of 31 DOAC samples (77%) across the DOAC treatment concentration range (Figure 1D).

The high anticoagulation levels were reflected in the ST Genesia DrugScreen results. ST Genesia DrugScreen failed to give valid results in 7 of 42 samples (17%), all of which had a high anticoagulation level: two LMWH samples (anti-Xa ≥ 0.5 IU/mL), three warfarin samples (INR ≥ 4.5), and two dabigatran (187 and 195 ng/mL) samples. The lag time and time to peak were prolonged at high DOAC levels, while the peak values were lower. However, the ETP remained essentially unchanged, even at anticoagulant levels high above the target range; that is, DOAC concentrations >400 ng/mL or apixaban concentrations of 428 ng/mL and 795 ng/mL, respectively.

3.3 | Comparing ST Genesia DrugScreen with the CAT assay

To compare the CAT assay with ST Genesia, we studied 24 samples using the CAT 5 pM TF assay. ST Genesia failed to provide results in three samples with high anticoagulant concentration (Table 2), while CAT failed in one case involving dabigatran due to a calibration error that may have been related to the alpha-2-macroglobulin concentration. Ultimately, 20 samples were available for both assays: 14 activated factor X (FXa) inhibitor samples (nine rivaroxaban, five apixaban), four warfarin samples, and two LMWH samples (Figure 3).

The sensitivity of lag time, time to peak, peak height, and ETP raw values differed among the assays, with ST Genesia showing higher ETP and peak and shorter lag time than CAT (Figure 3). In ST Genesia, only 6 of 21 samples (29%) had diminished ETP: two rivaroxaban samples, a heparin pooled sample, and three warfarin pooled samples (Figure 3D). In contrast, ETP was normal in only 2 of 21 samples analyzed using CAT (10%), both of which were rivaroxaban samples. These samples also showed normal ETP in ST Genesia (Figure 3D).

Lag time was the most sensitive variable, regardless of the TG method used; it was prolonged in all samples analyzed using CAT and in 20 of 21 samples (95%) analyzed using ST Genesia (Table 2, Figure 3A). All samples showed prolonged time to peak when analyzed using ST Genesia; 19 of 21 (90%) did so when analyzed using CAT (Figure 3B). The peak value was normal in 1 of 21 samples (5%) analyzed using ST Genesia, while 3 of 21 (14%) were normal with CAT (Figure 3C).

The overall correlations between ST Genesia and CAT methods were poor, as follows: lag time, $R^2 = 0.19$; time to peak, $R^2 = 0.20$; peak height, $R^2 = 0.20$; ETP, $R^2 = 0.20$. Such poor correlation reflects the mode of action of individual anticoagulants, which were correlated with drug concentrations in a drug-specific manner. For rivaroxaban, the lag time showed an R^2 value of 0.43, time to peak

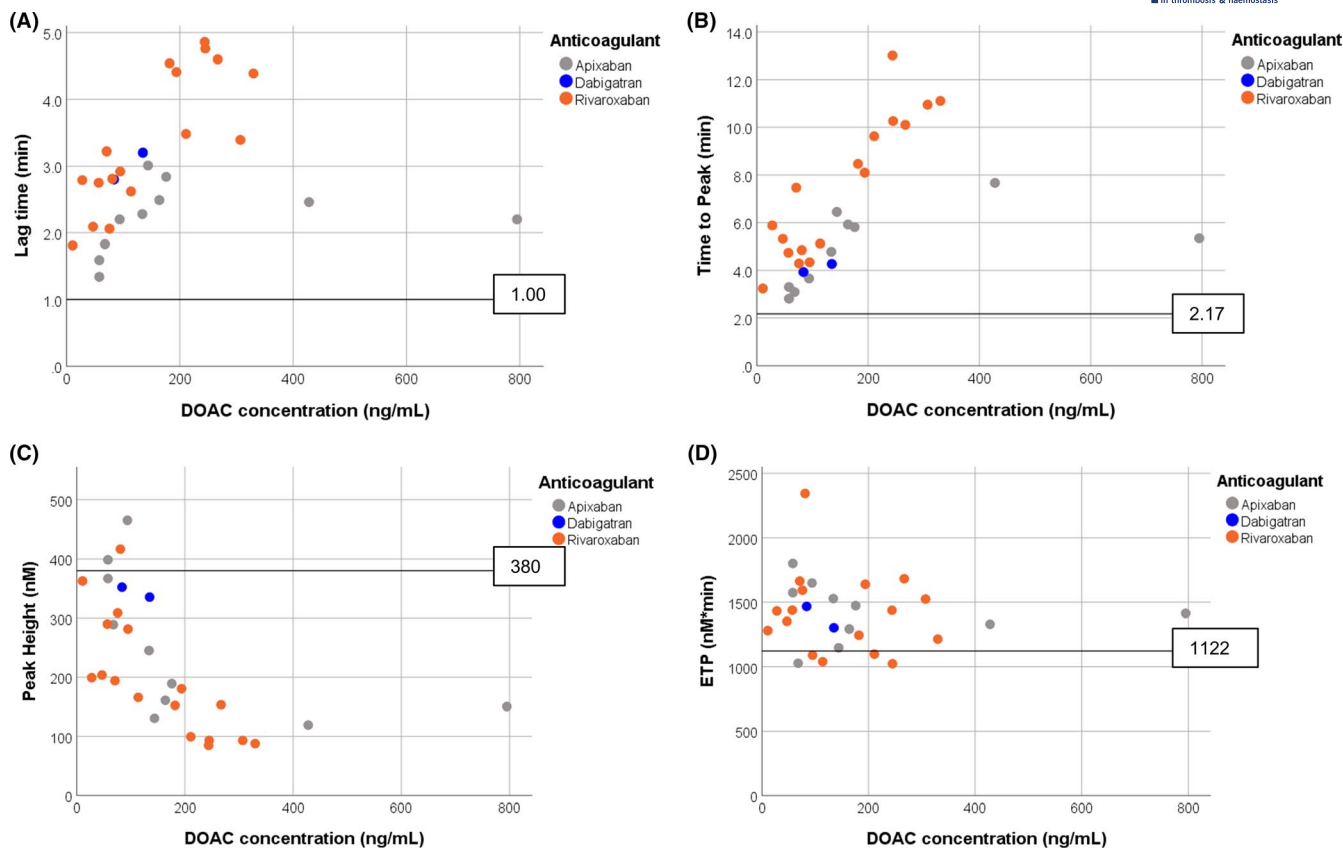


FIGURE 1 DOAC response in 31 samples as assessed using ST Genesia DrugScreen. Increasing DOAC concentrations were correlated with (A) prolonged lag time ($R^2 = 0.33$) and (B) greater time to peak ($R^2 = 0.53$), and (C) lower peak ($R^2 = 0.45$). (D) ETP responses varied widely ($R^2 = 0.08$). The line shows the reference values reported in the literature.⁶ DOAC, direct oral anticoagulant; ETP, endogenous thrombin potential

an R^2 value of 0.27, peak height an R^2 value of 0.001, and ETP an R^2 value of 0.11; as such, no correlations were detected. In contrast, for apixaban, lag time showed an R^2 value of 0.65, time to peak an R^2 value of 0.92, peak height an R^2 value of 0.97, and ETP an R^2 value of 0.76, delineating previous discrepancies with these two factor Xa inhibitors.¹¹

The differences in responses were calculated by plotting each result variation against the mean of the results (Bland-Altman). Based on lag time change, CAT seemed more sensitive to all the studied anticoagulants, especially rivaroxaban, which was the DOAC with the highest observed concentrations. In all, ST Genesia yielded more prolonged lag time and time to peak than CAT, while peak height and ETP were higher, indicating a slower initiation phase but more capacity to generate thrombin.

4 | DISCUSSION

To our knowledge, this was the first study to compare the new ST Genesia DrugScreen thrombin generation method with the established CAT assay in plasma samples of anticoagulated patients. Since the DrugScreen is the preferred assay for assessing anticoagulant activity according to Stago, we chose this reagent to compare with CAT 5 pM TF concentration, which is commonly used to assess

anticoagulated samples.^{11,12} When 5 pM TF is used, also factors VIII and IX contribute to TG.¹³ In addition, our small pilot study tested the Stago ThromboScreen reagent, which is close in TF concentration to the CAT 5 pM assay. The results showed very modest responses in some strongly anticoagulated patients. Overall, the results differ between the assays. CAT seemed more robust and provided results even when anticoagulation levels exceeded their clinical or assumed targets.

The ST Genesia DrugScreen results correlated with the concentrations of DOACs, especially when the FXa inhibitor apixaban was used, the peak was decreased, or the lag time was prolonged. This is in concordance with a recent study.⁷ The level of anticoagulation impacted the findings; the difference between the methods grew at anticoagulant levels that were higher than the target (Table 2). The peak values for rivaroxaban—approximately 100 nM with a rivaroxaban concentration of 200 ng/mL—were comparable to the data previously reported using the ThromboScreen reagent.¹⁴ Indeed, in LMWH and warfarin samples, the DrugScreen was oversensitive to the anticoagulant levels exceeding the usual clinical targets (anti-Xa of ≥ 1.0 ; INR of ≥ 4.5), while CAT still provided readouts at these levels. In addition, in two high samples containing high dabigatran levels (187 and 195 ng/mL), DrugScreen did not provide readouts. In the case of rivaroxaban and apixaban, DrugScreen seemed to provide results at higher concentrations than with dabigatran, even at FXa inhibitor

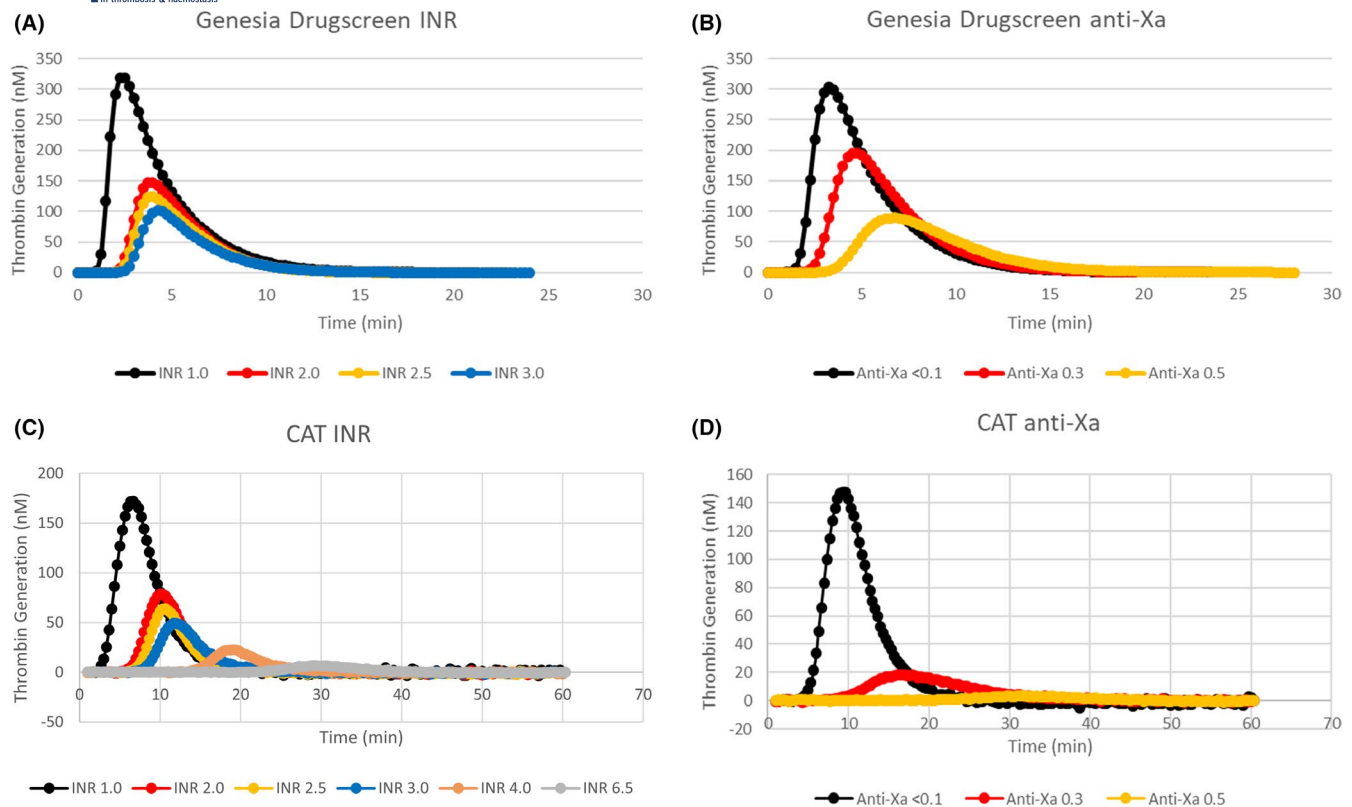


FIGURE 2 Thrombin generation profiles using ST Genesis DrugScreen and Calibrated Automated Thrombogram (CAT) in warfarin and heparin samples. ST Genesis DrugScreen and CAT exhibited trends of diminishing thrombin generation (TG) with increasing anticoagulation, although the magnitude of TG differed. ST Genesis DrugScreen did not provide results in samples with anticoagulant levels with an international normalized ratio (INR) >3.0, whereas CAT yielded results at INRs up to 6.5

concentrations of 330 and 795 ng/mL, respectively. In only one apixaban sample, the drug level was supratherapeutic, so distinctions at levels of overanticoagulation remain to be elucidated in the future.

Because the curves are flat, the DrugScreen reagent cannot be used to diagnose high warfarin, LMWH, or dabigatran levels, which is a clinical problem. Bleeding risks with warfarin increase by 8- to 10-fold at INRs >4.5 and at anti-Xa levels >0.7 IU/mL, which are considered supratherapeutic in patients with trough levels of LMWH.^{15,16} These differences are highly clinically relevant after incidents such as a severe bleeding episode, impaired renal function, or thrombolysis, as well as during unfractionated heparin therapy and warfarin overdose assessment.

ST Genesis has been proposed as a tool to exclude the presence of DOACs, because it can detect levels <30–50 ng/mL.⁷ We were unable to confirm this finding because only two samples had low DOAC levels (<50 ng/mL). However, we did find that lag time was prolonged in all DOAC samples using DrugScreen. The peak value was also diminished in >95% of the samples studied. In accordance with a previous study, the ETP of DrugScreen was insensitive to DOAC effects,⁷ exhibiting mostly normal values. In contrast, the CAT ETP was decreased in most of the samples (86%). It is not possible to compare the two methods directly because the formula for calculating ETP in ST Genesis is not publicly available. Recently, ST Genesis was used to assess TG in patients with coronavirus disease 2019, but the effects of anticoagulation were eliminated *in vitro* before testing.¹⁷

Unlike previous TG methods, in which chromogenic reactions determine the readout,¹⁸ ST Genesis is based on a fluorogenic measurement similar to that of CAT. This likely leads to similar results between the two methods. Again, in a comparison between ST Genesis and the CAT assay using 12 normal samples, BleedScreen correlated with both 1 and 5 pM TF-triggered CAT results in terms of both lag time and peak, whereas ETP correlated poorly.¹⁹ In the present study, the overall correlations between the methods were poor because the different anticoagulants had different modes of action. In contrast, the apixaban concentrations correlated well, with R^2 values >0.90 for both lag time and peak height. Regardless of the anticoagulant, the ETP failed to correlate between the methods.

Differing TF concentrations between the methods likely explain some of the quantitative differences in the TG results. That is, ST Genesis DrugScreen has higher ETP and shorter lag time than CAT. Previous studies have shown that a TF concentration of 1 pM in CAT is sensitive to hypocoagulable conditions such as hemophilia,²⁰ while 5 pM TF generates more thrombin at baseline under hypercoagulable states. The difference in ETP between baseline and hypercoagulability is also enhanced at lower TF concentrations (1 pM).²¹ Based on these previous findings, we assume that the DrugScreen reagents contain TF levels >5 pM, but the actual concentration is not publicly available (Table 1).

The present study had several limitations. For example, no clinical information, such as indication for anticoagulation, were available

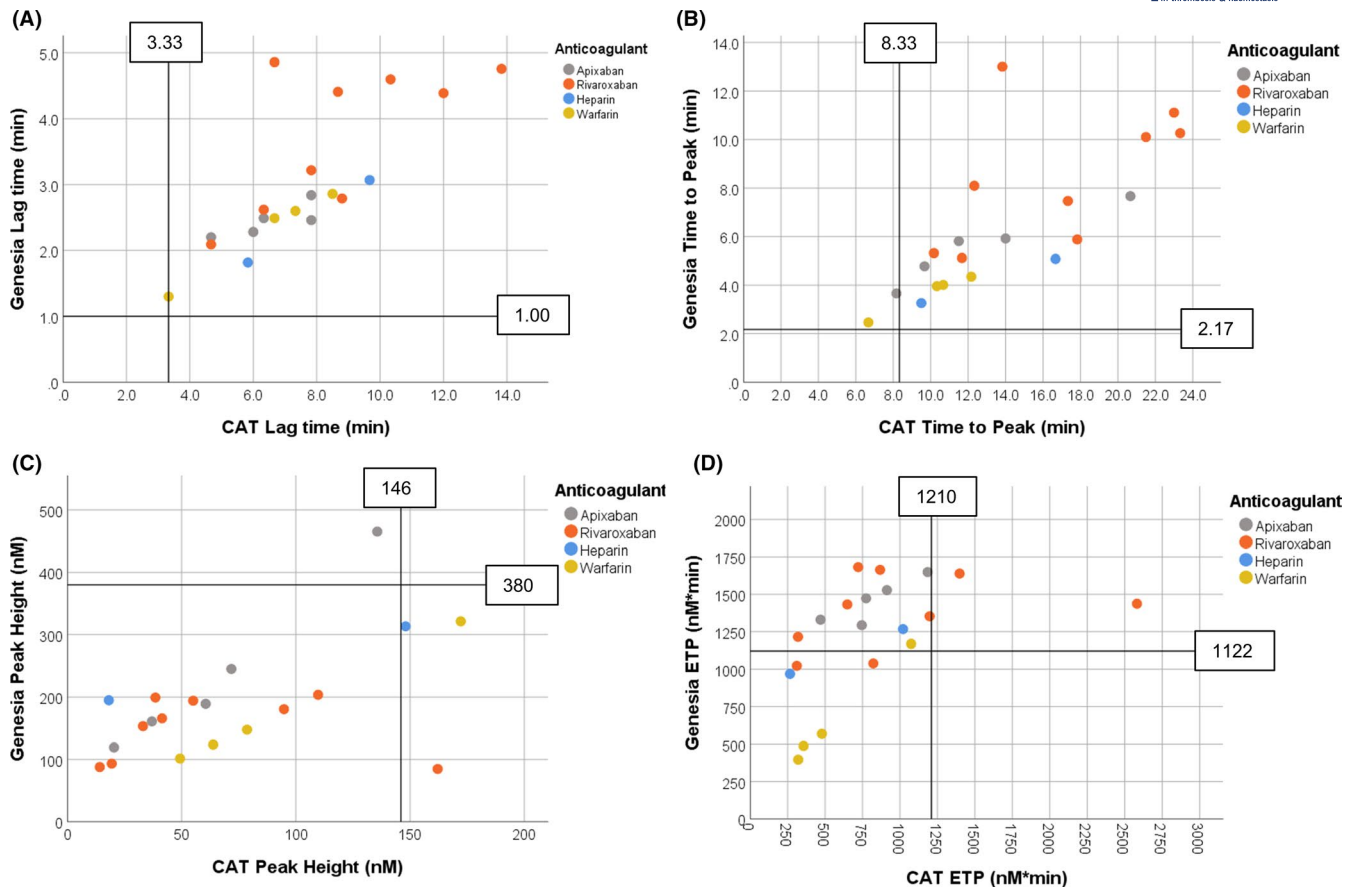


FIGURE 3 Thrombin generation in both ST Genesis DrugScreen and Calibrated Automated Thrombogram (CAT) assessed in 20 samples. The interpretation of the results differed somewhat in all studied parameters, but the endogenous thrombin potential (ETP) data showed complete discrepancy between the two methods. Lag time was the variable in which the samples were mostly abnormal in both assays. Lag time was prolonged in all samples using CAT and in 20 of 21 samples (95%) using ST Genesis (A). In terms of time to peak, all samples were abnormal using ST Genesis, while 19 of 21 (90%) were abnormal using CAT (B). Peak value was normal in one of 21 samples (5%; apixaban at 94 ng/mL) using ST Genesis and in 3 of 21 samples (14%) using CAT (C). Using ST Genesis, only 6 of 21 samples (29%) showed diminished ETP. In CAT, the ETP was abnormal in 19 of 21 samples (90%). Lines show the ST Genesis reference values reported in the literature⁶ and the CAT in pooled normal plasma

because the samples were collected and analyzed anonymously. Furthermore, the DOAC intake was unknown, and no other coagulation laboratory tests were carried out. Since some of the samples were obtained as surplus plasma from a thrombophilia panel, patients undergoing acute thrombosis may have been included, potentially confounding the results. The strength of the present study was that it used actual, real-life patient samples with a range of exposure to specific anticoagulants. Since INR and anti-Xa samples were pooled, individual differences in response to TG were dampened, enabling more accurate comparison of the methods. While the different TG capacities of the reagents impact quantitative TG variables, the use of reference intervals of the ST Genesis and CAT methods enabled the two methods to be compared.

Few studies have compared ST Genesis with the CAT assay. In a small study involving six patients who had undergone liver transplant with repeated sampling, the ThromboScreen and BleedScreen reagents exhibited lower ETP and peak values than 5 pM CAT. We detected opposite results using the DrugScreen, which showed higher peak and ETP values, perhaps because it has a high TF concentration and low

factor VII levels, which are common in liver disease. The lag time was shortest using the ThromboScreen reagent, while BleedScreen and CAT exhibited similar results.⁹ In the present study, DrugScreen exhibited a shorter lag time than CAT, as well as higher ETP and peak values. Another study by Roulet et al²² involving 85 patients with liver cirrhosis reported similar results. The main difference was reagent-specificity: The ThromboScreen reagent of CAT showed similar results to ST Genesis. A practical difference between these assays is the higher sample volume required by the ST Genesis (800 μ L; Table 1). Finally, in our study, while the absolute ST Genesis results displayed stronger TG (shorter lag time, higher peak and ETP), the CAT assay performed more robustly when patients were overly anticoagulated. Thus, the sensitivity differences between the reagents seemed nonlinear.

5 | CONCLUSIONS

Both the ST Genesis DrugScreen and CAT methods correlated with anticoagulation intensity; ST Genesis and CAT showed similar lag time

and peak thrombin alterations, while the respective readouts did not correlate well. ST Genesia DrugScreen exhibited weaker ETP response, possibly because of differences in the sensitivity of the reagents. As the ETP remained normal in most DOAC samples using DrugScreen, it should not be used to assess ETP in patients prescribed DOACs. However, the lag time and peak values behave as expected when using DrugScreen, offering better markers. At high INR and anti-Xa levels, the CAT method seemed more robust and provided readouts, unlike ST Genesia DrugScreen. Overall, ST Genesia DrugScreen remains limited in its practical use, failing to measure overanticoagulation using warfarin and heparin, as well as showing normal ETP with DOACs.

ACKNOWLEDGMENTS

Diagnostica Stago (France) is acknowledged for allowing access to use the ST Genesia device for this study. J. Perholehto, K. Karjalainen, J. Toikka, K. Salonen, and L. Tulikallio of the HUSLAB Coagulation Laboratory are thanked for their contribution in collecting the patient samples for this study.

AUTHOR CONTRIBUTIONS

TH, RL, and LJ-K were involved in the planning of the study, in analyzing the data, and in formulating and finalizing the manuscript. ML collected patient samples from the study and ran the analyses (CAT, ST Genesia, and DOAC concentrations). All authors approved the final version of the manuscript.

RELATIONSHIP DISCLOSURE

The authors declare that nonfinancial support was received from Diagnostica Stago (use of the ST Genesia device). The authors have no other disclosures to declare.

ORCID

Tuukka A. Helin  <https://orcid.org/0000-0002-5273-8088>

REFERENCES

- Al Dieri R, de Laat B, Hemker HC. Thrombin generation: what have we learned? *Blood Rev*. 2012;26:197-203.
- MacFarlane RG, Biggs R. A thrombin generation test; the application in haemophilia and thrombocytopenia. *J Clin Pathol*. 1953;6:3-8.
- Hemker HC, Giesen P, Al Dieri R et al. Calibrated automated thrombin generation measurement in clotting plasma. *Pathophysiol Haemost Thromb*. 2003;33:4-15.
- Dargaud Y, Wolberg AS, Gray E, Negrier C, Hemker HC. Subcommittee on Factor VIII. Proposal for standardized preanalytical and analytical conditions for measuring thrombin generation in hemophilia: communication from the SSC of the ISTH. *J Thromb Haemost*. 2017;15:1704-1707.
- Kintigh J, Monagle P, Ignjatovic V. A review of commercially available thrombin generation assays. *Res Pract Thromb Haemost*. 2018;2:42-48.
- Doux fils J, Morimont L, Bouvy C et al. Assessment of the analytical performances and sample stability on ST Genesia system using the STG-DrugScreen application. *J Thromb Haemost*. 2019;17:1273-1287.
- Pfreppe C, Metze M, Siegemund A, Klöter T, Siegemund T, Petros S. Direct oral anticoagulant plasma levels and thrombin generation on ST Genesia system. *Res Pract Thromb Haemost*. 2020;4:619-627.
- Helin TA, Lemponen M, Hjemdahl P, Rönquist-Nii Y, Lassila R, Joutsu-Korhonen L. From laboratory to clinical practice: dabigatran effects on thrombin generation and coagulation in patient samples. *Thromb Res*. 2015;136:154-160.
- Roulet S, Labrousche S, Freyburger G. Comparison of two thrombin generation methods, CAT and ST-Genesia, in liver transplant patients. *Thromb Haemost*. 2019;119:899-905.
- Hardy M, Douxfils J, Bareille M et al. Studies on hemostasis in COVID-19 deserve careful reporting of the laboratory methods, their significance, and their limitations. *J Thromb Haemost*. 2020;18:3121-3124.
- Helin TA, Virtanen L, Manninen M et al. Effects of thromboprophylactic doses of apixaban and rivaroxaban on coagulation and thrombin generation in association with total hip replacement. *J Thromb Thrombolysis*. 2017;43:562-569.
- Bloemen S, Hemker HC, Al Dieri R. Large inter-individual variation of the pharmacodynamic effect of anticoagulant drugs on thrombin generation. *Haematologica*. 2013;98:549-554.
- Hemker HC, Al Dieri R, De Smedt E, Béguin S. Thrombin generation, a function test of the haemostatic-thrombotic system. *Thromb Haemost*. 2006;96:553-561.
- Siguret V, Abdoul J, Delavenne X et al. Rivaroxaban pharmacodynamics in healthy volunteers evaluated with thrombin generation and the active protein C system: modeling and assessing interindividual variability. *J Thromb Haemost*. 2019;17:1670-1682.
- Palareti G, Leali N, Coccheri S et al. Bleeding complications of oral anticoagulant treatment: an inception-cohort, prospective collaborative study (ISCOAT). Italian Study on Complications of Oral Anticoagulant Therapy. *Lancet*. 1996;348:423-428.
- García DA, Baglin TP, Weitz JI, Samama MM. Parenteral anticoagulants: antithrombotic therapy and prevention of thrombosis, 9th ed: American college of chest physicians evidence-based clinical practice guidelines. *Chest*. 2012;141(1): e24S-e43S. <https://doi.org/10.1378/chest.11-2291>
- White D, MacDonald S, Edwards T et al. Evaluation of COVID-19 coagulopathy; laboratory characterization using thrombin generation and nonconventional haemostasis assays. *Int J Lab Hematol*. 2021;43(1):123-130.
- Devreese K, Wijns W, Combes I, Van Kerckhoven S, Hoylaerts MF. Thrombin generation in plasma of healthy adults and children: chromogenic versus fluorogenic thrombogram analysis. *Thromb Haemost*. 2007;98:600-613.
- Calzavarini S, Brodard J, Quarroz C et al. Thrombin generation measurement using the ST Genesia Thrombin Generation System in a cohort of healthy adults: normal values and variability. *Res Pract Thromb Haemost*. 2019;3:758-768.
- Chantarangkul V, Clerici M, Bressi C, Giesen PL, Tripodi A. Thrombin generation assessed as endogenous thrombin potential in patients with hyper- or hypo-coagulability. *Haematologica*. 2003;88:547-554.
- Machlus KR, Colby EA, Wu JR, Koch GG, Key NS, Wolberg AS. Effects of tissue factor, thrombomodulin and elevated clotting factor levels on thrombin generation in the calibrated automated thrombogram. *Thromb Haemost*. 2009;102:936-944.
- Talon L, Sinegre T, Lecompte T et al. Hypercoagulability (thrombin generation) in patients with cirrhosis is detected with ST-Genesia. *J Thromb Haemost*. 2020;18:2177-2190.