

# Thrombin generation for monitoring hemostatic therapy in hemophilia A: A narrative review

Marieke J. A. Verhagen<sup>1,2</sup>  | Lars L. F. G. Valke<sup>1,2</sup>  | Saskia E. M. Schols<sup>1,2</sup> 

<sup>1</sup>Department of Hematology, Radboud University Medical Center, Nijmegen, The Netherlands

<sup>2</sup>Hemophilia Treatment Center, Nijmegen-Eindhoven-Maastricht, Nijmegen, The Netherlands

## Correspondence

Saskia Schols, Department of Hematology, Radboud University Medical Centre, PO Box 9101, 6500 HB Nijmegen, the Netherlands.  
Email: saskia.schols@radboudumc.nl

## Abstract

Patients with severe hemophilia A (HA) have an increased risk of spontaneous and trauma-related bleeding because of a congenital absence of factor VIII (FVIII). Most severe HA patients use prophylactic FVIII concentrate, the effect of which can be monitored with FVIII activity level measurement. However, FVIII activity level is less valuable in predicting the potential clinical bleeding risk. Some patients still experience breakthrough bleeds despite adequate FVIII trough levels, whereas others do not bleed with trough levels below threshold. This difference may be caused by inter-individual differences in pro- and anticoagulant factors, the so-called hemostatic balance. Thrombin generation assays (TGAs) measure the hemostatic balance as a whole. Thereby, the TGAs may be a better tool in the guidance and monitoring of treatment in HA patients. In addition, TGAs offer the opportunity to determine the response to bypassing agents and treatment with non-factor replacement therapy, in which FVIII activity assays are not suitable for monitoring. This review summarizes the current knowledge about monitoring different HA treatment modalities by TGA, as a single treatment option and when used in a concomitant fashion.

## KEYWORDS

concizumab, emicizumab, factor VIII, hemophilia A, thrombin generation

## 1 | INTRODUCTION

Hemophilia A (HA) is characterized by an abnormal low coagulation factor VIII (FVIII) activity level. HA patients with severe (FVIII activity level <1%) disease have an increased risk of trauma-related and spontaneous bleeding, especially in joints and muscles. Prophylactic replacement therapy with FVIII concentrate is recommended for these patients to prevent bleeding and to preserve musculoskeletal

health.<sup>1</sup> The prophylactic scheme is now based on patient body weight and individual pharmacokinetic (PK) studies. Remarkably, there is a large inter-individual variability in PK dosing based on this algorithm.<sup>2</sup> Interestingly, however, some patients still experience breakthrough bleeds despite adequate FVIII trough levels, whereas others do not bleed with trough levels below threshold.<sup>3</sup> In addition, large heterogeneity in bleeding phenotype between patients with the same baseline FVIII activity level is observed.<sup>3-5</sup>

Manuscript handled by: Flora Peyvandi

Final decision: Flora Peyvandi, 10 January 2022

Marieke J.A. Verhagen and Lars L.F.G. Valke contributed equally.

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.

© 2022 The Authors. *Journal of Thrombosis and Haemostasis* published by Wiley Periodicals LLC on behalf of International Society on Thrombosis and Haemostasis.

The underlying difference in response to replacement therapy and bleeding phenotype may be caused by inter-individual differences in pro- and anticoagulant factors. This interplay can be measured with a thrombin generation assay (TGA) because it measures the hemostatic balance as a whole. Previous research has shown that the thrombin generation (TG) profile correlates better with bleeding phenotype than FVIII activity levels.<sup>3-7</sup> Therefore, response to treatment may be measured more adequately when incorporating individuals' TG profile alongside FVIII activity level. In addition, TGAs offer the opportunity to determine the response to bypassing agents (BPAs) and treatment with non-factor replacement therapy like emicizumab and concizumab, where traditional FVIII activity assays and coagulation bleeding times, like the activated partial thromboplastin time (APTT) and prothrombin time, cannot.

In recent years, studies have become available that investigated the role of TGAs in the monitoring of HA patients, especially in specific HA patient categories, like patients with inhibitors, patients on non-factor replacement therapy and FVIII pharmacodynamic (PD) monitoring. This paper aims to generate an overview of current knowledge about hemostatic monitoring of different HA treatment modalities with TGAs.

## 2 | SEARCH STRATEGY

A systematic search was undertaken in PubMed, Embase, and the Cochrane library from origin until 22 September 2021. We used the search terms "hemophilia A," "factor VIII," and "thrombin generation" in various configurations. The search strategy and inclusion and exclusion criteria are shown in detail in the Supplementary Appendix. Only articles in English were included and conference abstracts were excluded. Two authors (M.V. and L.V.) independently screened the articles for relevance. Discrepancies were resolved after discussion and consensus with the third author (S.S.). Furthermore, relevant articles from the reference lists of included articles were identified. The flow diagram of selected studies is shown in Figure 1.

## 3 | THROMBIN GENERATION ASSAYS

The calibrated automated TGA was developed by Hemker et al.<sup>8</sup> It is widely used in research and it is finding its way into clinical applications.<sup>9</sup> The hallmark of this assay is activation of TG by tissue factor (TF), in the presence of calcium and phospholipids, and measurement of the proteolytic activity of thrombin on added fluorescent

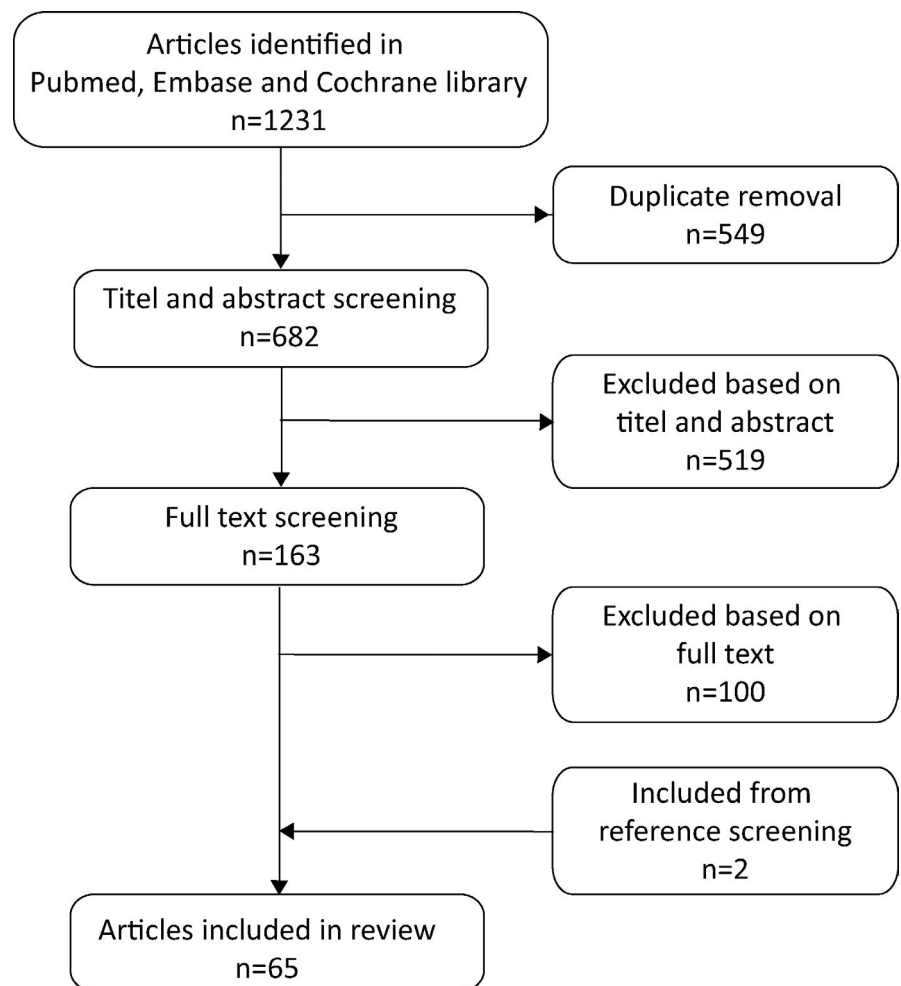
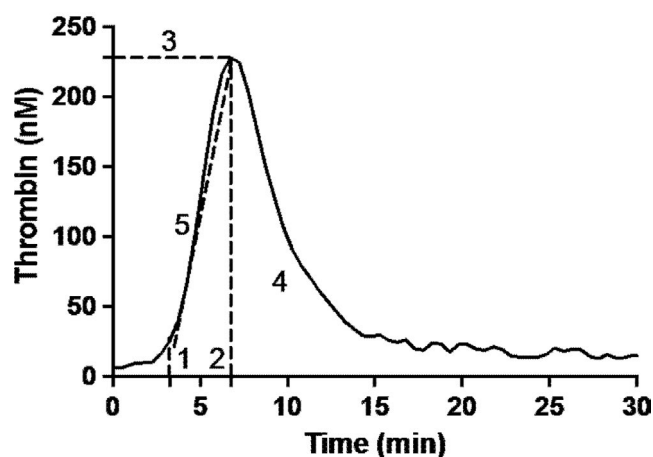


FIGURE 1 Flow diagram of studies included in the review

## Normal thrombin generation curve



**FIGURE 2** Normal thrombin generation curve with parameters. Example of a normal thrombin generation curve. The classic parameters of the thrombin generation are depicted with the numbers 1 to 4, in which: (1) lag time, the time until the thrombin generation signal increases with two standard deviations from baseline; (2) time to thrombin peak represents the time until the thrombin peak is reached; (3) thrombin peak height (TPH) is the maximum amount of thrombin formed; (4) (endogenous) thrombin potential ((E)TP), which is calculated as the area under the thrombin generation curve (AUC). Additionally, (5) represents the velocity of thrombin generation and is calculated by dividing the TPH by the time between the lag time and time to thrombin peak

substrate.<sup>10</sup> Today, multiple TGAs are commercially available, and their differences have been reviewed previously.<sup>11</sup>

Classically, four essential parameters can be obtained in the TG profile (Figure 2, numbers 1–4).<sup>8</sup> The lag time of TG is the time until the TG signal increases with two standard deviations from baseline. Afterwards, TG shows a steep rise that reaches its peak when thrombin breakdown equals thrombin formation. At normal antithrombin activity, thrombin peak height therefore reflects the velocity of net thrombin concentration that appears. The thrombin peak height (TPH) (i.e., the maximum amount of thrombin formed) is associated with the time to thrombin peak (the time until the thrombin peak is reached). The most important parameter of the TGA is the (endogenous) thrombin potential, which is calculated as the area under the TG curve.

An additional parameter, the velocity of TG (number 5 in Figure 2) gives an impression of the rate of TG over time. Whereas the ETP only shows the total amount of thrombin formation, the velocity of TG reflects the amount of thrombin that is generated in the acceleration phase. It is calculated by dividing the TPH by the time between the lag time and time to thrombin peak. It is suggested to have a better correlation with FVIII activity level than ETP because it represents the coagulation capacity to generate a sufficient amount of thrombin in a short period to stop a bleeding.<sup>12</sup>

In addition to conventional TGAs, three assays were developed that not only measure TG, but also plasmin generation,<sup>13–15</sup> thereby assessing the fibrinolytic capacities of the hemostatic system. Two

of these assays measure TG and plasmin generation in two separate wells.<sup>14,15</sup> The other measures both pathways simultaneously in only one well, which gives a better impression of the interaction of thrombin and plasmin generation as it happens *in vivo*.<sup>13</sup> A full discussion of plasmin generation and the clinical implications reaches beyond the scope of this review.

The Scientific and Standardization Committee of the International Society of Thrombosis and Haemostasis has given guidance on the use and interpretation of TGAs.<sup>16</sup> In this, several aspects need to be taken into account, like the use of corn trypsin inhibitor (CTI).<sup>17,18</sup> Some authors state that the addition of CTI, which inhibits contact pathway inhibition, is only necessary at low concentrations of TF ( $\leq 1$  pM).<sup>19</sup> However, others have shown that TG in HA patients with low FVIII activity levels is not affected by the addition of CTI.<sup>17</sup> Possibly, CTI addition only decreases inter- and intra-assay variation at low TF levels and prevents formation of a plateau phase at supra-therapeutic FVIII activity levels ( $>140$  IU/dl).<sup>19</sup>

The amount of TF added determines the pathway of TG. A low concentration ( $\leq 1$  pM) of TF is most suitable to detect hypocoagulability because of FVIII or FIX deficiency and to monitor treatment with factor concentrate.<sup>16</sup> Higher TF concentrations do not show advantages except a lower inter- and intra-assay variation, but instead show a lower discriminating ability at low FVIII activity levels and can cause substrate depletion in combination with BPAs.<sup>20,21</sup>

Platelet rich plasma (PRP) or platelet poor plasma (PPP) can both be used in TGAs, where it is important to adhere to preparation guidelines.<sup>16</sup> Because of the possibility of freezing PPP and determine TG in batches instead of directly after sample collection as with PRP, it is used more frequently in studies. However, PRP may show a more realistic TG because platelets are an essential part of hemostasis and these assays could optimize FVIII supplementation therapy.<sup>22</sup> Last, to optimize standardization and comparability between centers, a standard pooled plasma should be run along every TGA as positive control. Results can then be reported as percentage of normal pooled plasma, to reduce inter-center assay variability and to improve comparability between studies.<sup>16,23</sup>

## 4 | FACTOR VIII CONCENTRATE

Multiple studies have shown a strong correlation between FVIII activity level and TG parameters (except for lag time).<sup>4,5,21,24–28</sup> Despite these correlations, a wide inter-patient variability in TG was observed when measured at different time points following a standard infusion of FVIII concentrate.<sup>4,21,24</sup> However, the intra-patient variability was small, resulting in a predictable relation between FVIII and TG for an individual patient.<sup>24</sup> The inter-patient variability in TG is likely a result of differences in PK and variation in the individual response of TG to FVIII therapy.<sup>24</sup> The study by van Veen et al. showed that some patients can generate normal TPH or ETP with subtherapeutic FVIII activity levels, whereas other patients have a low TG profile despite normal FVIII activity levels.<sup>21</sup>

Several studies reported a prolonged duration of TG response after administration of FVIII. This response of TPH and ETP sustained, even though FVIII activity levels declined over time.<sup>4,24,27,29</sup> Once again, this prolonged TG effect was variable between patients.<sup>4,24</sup> The wide inter-patient variability could be explained by variations in pro- and anticoagulation factors other than FVIII.

Even though these studies suggested that TGAs may be clinically useful, they failed to show clinical predictive value on bleeding risk after FVIII administration. The relationship between TG parameters and the clinical bleeding phenotype was investigated by Dargaud et al. Their results were based on data of the GENA-21 study, in which severe HA patients were treated with Nuwiq (human-cll rhFVIII). At start, a combined PK/PD study was performed and patients were treated with a personalized prophylactic regime, based on the FVIII PK data. Bleeding events were reported in patient diaries. Plotting the results of the PK study against time after FVIII concentrate infusion showed that there was no difference in FVIII activity level between patients with and without spontaneous bleeding. However, when TG was measured, patients with spontaneous bleeding showed a significant lower ETP compared with patients without spontaneous bleeding episodes at every time point after infusion.<sup>3</sup>

In line with this study, Delavenne et al. investigated if prophylactic FVIII dosing could be improved by incorporating ETP alongside FVIII trough levels in a PK/PD-dosing model. The authors used the sigmoid Emax model to describe the data and added individual PK/PD Bayesian estimation to improve the predictive performance. Generated data contributed to simulate the effect of different dosing strategies on annual bleeding rate (ABR). Patients were divided into three groups according to baseline ETP. As expected, patients with the highest baseline TG (600 nmol/L-min) had the lowest ABR (0.88 [0.42–1.87]), even with the lowest dosage of FVIII replacement therapy (20 IU/kg every 4 days). Although patients with the lowest third of baseline ETP (200 nmol/L-min) still had a higher ABR (1.66 [0.84–3.57]) with the highest dosage and the shortest dosing interval (60 IU/kg every 2 days), thereby indicating that personalized FVIII dosing of this specific FVIII concentrate according to ETP is beneficial to reduce bleeding in patients with low TG.<sup>30</sup>

Besides the previously described *ex vivo* studies, several spiking studies were published. In these *in vitro* studies, plasma from HA patients (without inhibitors) was spiked with FVIII concentrate and TG was measured. In one study, plasma of severe HA patients was spiked with 0.5 and 1.0 IU/ml plasma-derived FVIII (pdFVIII) concentrate. TG reached a plateau phase with a FVIII activity level >50% of normal, indicating that FVIII activity level was no longer the limiting factor.<sup>5</sup> This plateau phase in TG was confirmed by others, with a plateau reached at FVIII activity levels ranging from 20% to 50%.<sup>22,31,32</sup>

However, only one spiking study showed a linear, dose-dependent response of TG to increasing dosage of FVIII concentrate in plasma of 10 severe HA patients. This difference can be caused by the variability in TGAs and the specific FVIII products used for these experiments. In this study, the FVIII activity level at which TG

was normalized showed a major variation in individual patients, indicating the need for an individualized dosage of FVIII concentrate.<sup>33</sup>

## 5 | BYPASSING AGENTS

TGAs are also described in studies for monitoring BPAs in HA patients with inhibitors, like activated prothrombin concentrate (aPCC) and activated recombinant FVII (rFVIIa). aPCC is a multicomponent concentrate that contains activated and nonactivated vitamin K-dependent coagulation factors. It has multiple modes of action, including the formation of the prothrombinase complex, resulting in TG (Figure 3A, number 3). rFVIIa is a single-component concentrate that binds with TF and activates FX (Figure 3A, number 2). Furthermore, rFVIIa activates FX, independent of TF, on the surface of activated platelets. Because of the complexity of their mechanisms of action and their influence on coagulation bleeding times, no routine laboratory test is suitable to monitor treatment with BPAs. Since the final product generated by a BPA is thrombin, it seems reasonable to measure TG to monitor treatment. Because the effect of rFVIIa is platelet dependent, PRP is most suitable to be used when measuring TG.<sup>18,34</sup> Dargaud et al. showed that the use of PPP underestimated hemostatic efficacy of rFVIIa by approximately 30%.<sup>34</sup>

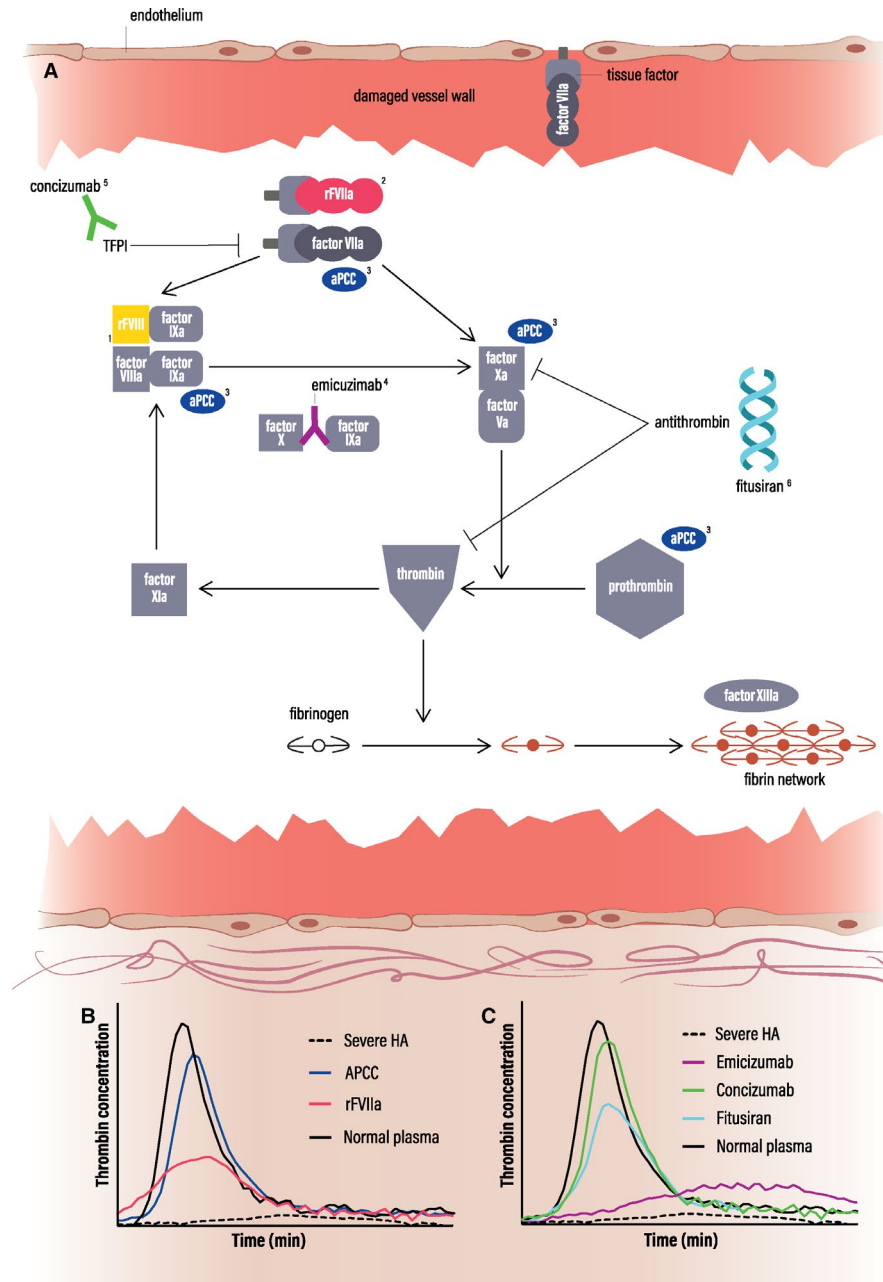
### 5.1 | Effect of aPCC on thrombin generation

Throughout the years, several studies reported that TGAs can measure the PK/PD properties of BPAs. Various studies spiked inhibitor plasma *in vitro* with increasing amounts of aPCC and measured TG afterwards. ETP and TPH were normalized with concentrations of 1–2 IU/ml aPCC, which corresponds to the usual therapeutic dose of 50–100 IU/kg<sup>35,36</sup> (Figure 3B). Subsequently, to investigate the pharmacodynamics of aPCC, TG was measured at different timepoints after infusion of 65–100 IU/kg aPCC in three HA inhibitor patients. In all patients, maximum TPH was achieved 15–30 min postinfusion. The total amount of TG halved between 4 and 7 h, which is in accordance with the clinical treatment strategy of aPCC (i.e., 50–100 IU/kg two or three times a day in case of bleeding).<sup>35</sup>

The hemostatic effects of long-term prophylactic aPCC were monitored every 3–6 months in seven pediatric HA patients with an inhibitor after they failed immune tolerance induction therapy. TG was determined at trough level and 15–30 min postinfusion (60–100 IU/kg aPCC). In most patients, ETP was restored to 80% of normal after infusion of aPCC. Mean trough ETP was 2.6-fold higher in patients receiving aPCC prophylaxis compared with control inhibitor plasma.<sup>37</sup>

### 5.2 | Effect of rFVIIa on thrombin generation

In addition to the observed increase in TG with aPCC, the effect of rFVIIa on TG is less abundant (Figure 3B). In a study with five severe



**FIGURE 3** Schematic impression of the coagulation cascade with the treatment modalities for hemophilia A and their impact on the thrombin generation profile. (A) Plasma-derived or recombinant FVIII (rFVIII) (1) replaces the deficient FVIII, which together with FIXa can lead to activation of FX. Recombinant FVIIa (rFVIIa) (2) binds with tissue factor (TF) to form the TF/rFVIIa-complex to activate FX. The TF/rFVIIa complex also activates FIX, which (with cofactor VIIIa) leads to further activation of FX. Activated prothrombin complex concentrate (aPCC) (3) contains the vitamin K-dependent coagulation factors II, VIIa, IX, and X. Emicizumab (4) replaces the function of FVIII and binds FIXa and FX to activate FX. Concizumab (5) inhibits the function of tissue factor pathway inhibitor (TFPI). Fitusiran (6) inhibits the function of antithrombin. (B) The impact of rFVIIa and aPCC on the thrombin generation profile. The curve of rFVIIa was obtained in platelet poor plasma (PPP), which was spiked with rFVIIa at a corresponding concentration of 270  $\mu\text{g}/\text{ml}$ . aPCC was spiked in PPP in a corresponding dosage of 100 IU/kg. (C) The impact of emicizumab, concizumab, and fitusiran on the thrombin generation profile. The thrombin generation curve of emicizumab was obtained in PPP from a patient who was treated with 1.5 mg/kg emicizumab weekly (after four loading dosages). The thrombin generation curves of concizumab (0.15 mg/kg, daily) and fitusiran (80 mg subcutaneously, monthly) were also obtained in PPP

HA patients with an inhibitor, TG was measured before and after the administration of 90  $\mu\text{g}/\text{kg}$  rFVIIa. TPH increased and reached maximum levels at 30–60 min. They remained significantly elevated over a 4-h period, reflecting the average anticipated half-life of rFVIIa

(3–4 h). A strong within-patient correlation was found for TPH and rFVIIa levels. However, TPH was only restored to 20% of normal.<sup>38</sup>

Furthermore, two studies evaluated the TG response after administration of a conventional rFVIIa dose (i.e., three times 90  $\mu\text{g}/\text{kg}$ )

and a single high dose (i.e., 270 µg/kg). A single dose of 270 µg/kg reached higher TG, whereas multiple doses of 90 µg/kg resulted in a more sustained TG.<sup>39</sup> Both interventions decreased lag time and time to peak and increased TPH and ETP, but all values remained below the reference range. In patients with a good clinical response, lag time and time to peak were shorter and TPH and ETP were greater than in the poor response group.<sup>40</sup>

In a crossover study, six inhibitor patients received a bolus injection of 75 IU/kg aPCC alternated with 90 µg/kg rFVIIa. TG was measured predose and at several timepoints afterwards. ETP and TPH exceeded normal controls after infusion with aPCC. However, TG parameters did not reach normal values after administration of rFVIIa. ETP and TPH were almost two-fold greater following aPCC infusion compared with rFVIIa.<sup>41</sup>

Dargaud et al. showed that TG profiles obtained with *in vitro* and *ex vivo* experiments are comparable.<sup>34</sup> In a follow-up study on the performance of TGAs to guide the therapeutic choice of the most effective BPA and dosage, 6 patients were investigated with severe HA and high-titer inhibitors undergoing 10 invasive procedures in total. A three-step protocol was used to individually tailor BPA therapy: (1) *in vitro* spiking experiments with increasing concentrations of rFVIIa and aPCC to determine the minimum dose that normalizes TG capacity; (2) *ex vivo* TG measurement before and after administration of the most effective BPA; and (3) monitoring the chosen BPA during surgery and the postoperative period. The results of the *ex vivo* step were consistent with the results of the *in vivo* spiking experiments and TG monitoring perioperatively. In patients with normalized ETP, no bleeding complications occurred.<sup>42</sup>

The only study that failed to support the finding that the TGA is a useful tool to monitor treatment was by Mancuso et al. In this study TG was measured in HA patients with inhibitors in the surgical setting. They showed that 30 min after a preoperative bolus, TG increased significantly. However, this increase was completely lost 30 min after a second bolus postoperatively. Interestingly, no bleeding occurred in patients with persistently impaired TG postoperatively.<sup>43</sup> This phenomenon can possibly be explained by the consumption of the administered clotting factors during surgery.

In recent years, several new BPAs are developed and the ability of the TGA to measure pharmacodynamic properties was established for these new treatment modalities. Examples of these new agents include PEGylated liposome-formulated FVIIa,<sup>44</sup> MC710 (a plasma derived FVIIa and FX mixture),<sup>45</sup> marzeptacog alfa (an rFVIIa with four amino acid substitutions),<sup>46</sup> and MOD-5014 (a long-acting carboxy-terminal peptide-modified FVIIa).<sup>47</sup>

## 6 | EMICIZUMAB

Emicizumab is a bispecific FVIII-mimicking antibody that forms a pseudo-tenase complex with activated FIX (FIXa) and FX to overcome FVIII deficiency in HA (Figure 3A, number 4). It is administered *via* subcutaneous injections and has a long half-life of approximately 30 days. In the first-in-human trial, it was discovered that

emicizumab shortens APTT in healthy volunteers and normalizes APTT in FVIII-neutralized plasma, even at subtherapeutic concentrations.<sup>48</sup> Therefore, APTT and human FVIII based clotting assays were unable to measure the hemostatic effect of emicizumab.<sup>49</sup> Furthermore, combination therapy cannot be monitored with FVIII assays, which led to the suggestion to use TGAs to monitor hemostatic effects of emicizumab and combination treatment modalities.<sup>49</sup>

### 6.1 | Effect of monotherapy emicizumab on thrombin generation

The first-in-human trial showed that emicizumab did not increase TPH in case of adequate FVIII activity levels. However, in plasma in which endogenous FVIII was neutralized by an antibody *ex vivo*, TPH showed a dose dependent increase. The highest dosage of 1 mg/kg emicizumab reached mean TPH of 192 and 186 nM in Japanese and white healthy volunteers, respectively. In contrast, mean TPH in normal plasma was 385 and 405 nM, respectively, indicating that emicizumab treatment increases TG in the presence of an inhibitor to half of normal<sup>48</sup> (Figure 3C).

In the HAVEN-1 study, adult HA patients with inhibitors were included and randomized to receive either emicizumab prophylaxis or no prophylaxis with on demand treatment.<sup>50</sup> The pharmacokinetics and pharmacodynamics were reported in a second article.<sup>51</sup> TPH was measured after four loading doses of emicizumab with a median of 108.8 nM (95% confidence interval [95% CI] 29.7–187.0). TG was sustained at 72 weeks of treatment with a TPH of 108.7 nM (95% CI 46.3–171.1). This corresponded with a TG of approximately 20%–30% of healthy volunteers. Another study found that TG parameters with emicizumab monotherapy showed an equivalence of a FVIII activity level of 10%–40%.<sup>52</sup> Also, emicizumab concentration had a linear correlation with TPH.<sup>51</sup>

In a series of articles/studies from Israel, the clinical outcomes and laboratory results from patients treated with emicizumab are reported.<sup>53–56</sup> In total, 107 patients were included in a real-world prospective cohort study, including 58 children and 17 patients older than age 50 years with cardiovascular risk factors.<sup>53,54</sup> Of the included patients, 53 did not experience any bleeds. Of the bleeds in children, 94% were trauma-related and 61% of the adult patients with bleeding experienced spontaneous bleeding. The authors did not observe any difference in TG between patients that experienced bleeding compared with those who did not. An explanation for this discrepancy was not given by the authors, but it was apparent that spontaneous bleeding was mostly experienced by older patients and in joints affected by hemophilic arthropathy. Traumatic bleeding in children can still occur despite treatment with emicizumab.<sup>53</sup> In this study, TG was low before initiation of emicizumab. During maintenance therapy, TG increased to levels that corresponded with 20%–30% of normal controls.<sup>53</sup> This level of corrected TG was also observed in another study.<sup>57</sup> Interestingly, it was concluded that both TPH and ETP were lower in HA patients with inhibitors than

without inhibitors. This difference was observed at 5 weeks after emicizumab initiation, but disappeared at 1 year of treatment.<sup>53</sup> Additionally, infants younger than 1 year showed significant lower TG compared with older children and adults<sup>53,55</sup> as a result of higher clearance of emicizumab. And last, patients who presented with a major bleeding had a significant lower emicizumab concentration at time measurement, which was associated with lower TPH and ETP. During follow-up, emicizumab and TG increased to levels comparable to other patients in the cohort.<sup>53,54</sup> The mechanism for this observation remains elusive. Possibly, it could indicate nonadherence to therapy or the application of emicizumab at the site of bleeding.

## 6.2 | Emicizumab in combination with different treatment modalities

Because emicizumab was first used in HA patients with inhibitors, most reports address the TG effects of emicizumab combined with BPAs. However, some articles report the effect of emicizumab combined with FVIII replacement therapy. Two case reports showed that TG could be used to monitor treatment of HA patients with low-titer inhibitors when emicizumab was used.<sup>58,59</sup> In a spiking study, HA plasma with emicizumab at a concentration of 50 ng/ml (the steady-state concentration observed in the HAVEN-1 study) was combined with a pdFVIII/von Willebrand factor (VWF) concentrate. As expected from previous studies, samples with emicizumab significantly showed improved TG profiles compared with FVIII-deficient plasma. However, when pdFVIII/VWF was added to plasmas with emicizumab, there was no difference observed in TG compared with plasma with only pdFVIII/VWF. This indicates that pdFVIII/VWF and emicizumab have non-additive effects in the absence of inhibitors and thereby do not increase the thrombotic complication risk.<sup>60</sup> In this study, plasma was also spiked with aPCC and rFVIIa. Although a therapeutic dose of rFVIIa showed an increase in TPH and ETP in plasma with emicizumab compared with plasma without rFVIIa, it remained within ranges observed in normal controls. On the contrary, a low dose of aPCC (25 IU/kg) already caused an exponential increase of TG in the presence of emicizumab, with TG values of multiple times the normal range.<sup>60</sup>

This observation is in accordance with the observation of the HAVEN-1 study in which five patients experienced thrombotic complications when emicizumab was combined with high doses of aPCC.<sup>50</sup> This led to a black box warning for emicizumab to prevent combination of high doses of aPCC during a longer period (<100 IU/kg per 24 h). Afterwards, several case reports were published that studied the efficacy of emicizumab and BPA dosage, which was determined with TGAs.<sup>61-66</sup> The most interesting study in this regard is by Kizilocak et al.<sup>67</sup> The authors included 11 HA patients with an inhibitor who were treated weekly with emicizumab for at least 6 weeks. Plasma was spiked with rFVIIa (calculated corresponding dosages of 30–300 µg/kg) and aPCC (corresponding with dosages of 5–100 IU/kg) and TG was measured. Mean normal control values in this study were 55.2 nM for TPH and 617 nM·min for

ETP. TG parameters at baseline (only emicizumab treatment) were 11.9 nM and 251 nM·min, respectively. TG was already restored to normal values after spiking with 5 IU/kg aPCC (TPH 52.5 nM; ETP 741 nM·min) and exceeded normal values with higher concentrations. A dosage of 75 IU/kg increased TPH and ETP to eight times normal values (426.4 nM and 2801 nM·min, respectively) and aPCC of 100 IU/kg increased TG above the detection limit for all but one patient. When emicizumab was combined with rFVIIa, it was shown that the highest dosage of 240 and 300 µg/kg reached normal TG, but did not exceed these values.<sup>67</sup> These observations are in concordance with a second study that revealed comparable TG parameters with a low dose (30 IU/kg) of aPCC in combination with emicizumab than in samples with 100–200 IU/kg aPCC before start of emicizumab.<sup>68</sup> Furthermore, a spiking study examining eptacog beta, a novel rFVIIa, in combination with emicizumab showed low TG parameters compared to control plasma, even with the highest dosage of eptacog beta.<sup>69</sup>

Important work to unravel the underlying mechanism of excessive TG in case of combined treatment of emicizumab with aPCC was performed by Hartmann et al.<sup>70</sup> In their spiking studies, a sequence identical to emicizumab was used alone and in combination with aPCC and rFVIIa, both in various concentrations. As comparable with the previous study, the lowest concentration of 0.05 IU/ml aPCC normalized TG, whereas higher concentrations caused an excess of TG with a TPH five times above the normal range. Again, rFVIIa did not cause excess TG. Furthermore, the separate components of aPCC were added to HA plasma with the sequence identical to emicizumab, which showed that FIX, and to a lesser extent, FIXa, caused the synergistic effect of emicizumab and aPCC.<sup>70</sup>

Compared with endogenous FVIII, emicizumab does not have an on/off switch, causing ongoing TG in the presence of trace amounts of FIXa. In HA, in which FVIII is the rate limiting step of tenase complex formation, emicizumab is present in a much higher concentration, making FIX concentration the rate-limiting step.<sup>71</sup> Therapy with aPCC in HA patients with inhibitors with emicizumab is still feasible if rFVIIa is unavailable or ineffective, but should be used with caution in a low dosage and preferably under monitoring of TG. For emicizumab-treated HA patients without inhibitors, the main therapy in case of bleeding is still FVIII concentrate. Because FVIII has a greater affinity to FIX/FX than emicizumab, an additional hemostatic effect is not expected.<sup>71</sup>

## 7 | ANTI-TISSUE FACTOR PATHWAY INHIBITORS

Tissue factor pathway inhibitor (TFPI) is a strong regulator of the initiation phase of coagulation. TF binds to FVII after vessel wall injury to form FVIIa, which forms the TF-FVIIa complex to activate FX to FXa. This causes thrombin formation and the activation of the extrinsic pathway to enhance TG. TFPI has three Kunitz binding domains, of which the Kunitz 1 domain inhibits the TF-FVIIa complex. The Kunitz-2 domain binds FXa to form the

TF-FVIIa-FXa-TFPI quaternary complex. The Kunitz 3 domain interacts with protein S and the prothrombinase complex. Together, these processes shut down the initiation phase. However, in HA patients the amplification phase is impaired, which causes diminished TG. TFPI inhibition improves TG by increasing FXa generation during the initiation phase.<sup>72</sup> However, only 10%–50% of TFPI is in a free form in plasma available, whereas the rest is bound to the endothelium. It is therefore unknown if and to what extent TGAs overestimate TFPI inhibition.<sup>73,74</sup>

In the past decade, multiple anti-TFPI agents have been developed. The aptamer BAX-499 showed indeed an increase in TG in hemophilic plasma,<sup>75</sup> but the development of this agent was discontinued because of increased bleeding episodes in the phase 1 trial.<sup>76</sup>

Two monoclonal antibodies were used in *in vitro* pharmacodynamic spiking studies.<sup>77,78</sup> Marstacimab, which only binds the K2 domain of TFPI, was able to improve TG. Marstacimab in a concentration of 5 nmol/L reached a TPH that corresponded with a FVIII activity level of 10% and a concentration of 100 nmol/L to 40%.<sup>77</sup> Befovacimab binds both the K1 and K2 domain of TFPI and showed a dose-dependent increase of TG until a concentration of 5 nmol/L, after which it reached a plateau phase. TG (both TPH and ETP) with befovacimab at a concentration of 5 nmol/L was comparable with FVIII activity level of 40%, which did not increase with higher concentrations of 10 and 100 nmol/L.<sup>78</sup>

Concizumab is a K2 domain-specific anti-TFPI monoclonal antibody (Figure 3A, number 5), of which most pharmacodynamic studies are available. First, *in vitro* spiking studies of hemophilic plasma showed a dose-dependent increase of TG, with a plateau of near normal TG at concentrations  $\geq 10$  nmol/L. Even in healthy subjects, concizumab increased TPH and ETP.<sup>79</sup> Second, in a dose-escalation study, concizumab was shown to increase TG, where the highest dose (0.8 mg/kg) was able to restore TG to normal values in the majority of patients. More importantly, when stratified for unbound TFPI compared with baseline it was found that almost all patients with  $< 25\%$  TFPI from baseline had normal TPH values<sup>80</sup> (Figure 3C). Last, the results from the phase 1/1b clinical trials were used to establish a PK/PD model to guide target dosing for the phase 2 clinical trial. TPH and plasma concizumab concentrations showed a strong correlation, and it was established that a concizumab concentration of  $> 100$  ng/ml could reach TG profiles within the normal range.<sup>81</sup> Indeed, in the subsequent phase 2 trial, all HA patients with a concizumab concentration  $> 100$  ng/ml showed TPH within the normal range.<sup>82</sup>

The additional effects of concizumab and BPAs were also investigated. Although emicizumab and aPCC showed an exponential increase in TG because of drug–drug interaction, concizumab and aPCC acted additive and showed only minimal drug–drug interaction. TG was normalized with aPCC in a concentration of 0.5 IU/ml in the presence of concizumab; higher doses of aPCC resulted in higher than normal TG. The same results were found when using rFVIIa and rFVIII,<sup>74</sup> thereby indicating that aPCC, rFVIIa, and rFVIII can be used concomitantly with concizumab, but dosage should be adjusted to reach a balance between effective hemostasis and safety, in which TG profiles can have an essential role.

## 8 | ANTITHROMBIN INHIBITION

Antithrombin inhibits the coagulation cascade by binding to FXa and thrombin. Patients with a genetically antithrombin deficiency are known to have a higher risk of venous thromboembolism, thereby providing the rationale to lower antithrombin in hemophilia patients. The effect of antithrombin lowering on TG was first shown in healthy volunteers and hemophilia patients without inhibitors. These studies used fitusiran subcutaneously, a small interfering RNA that decreases antithrombin production by the liver (Figure 3A, number 6). In hemophilia patients, TG increased depending on the percentage of antithrombin lowering. Patients reached TPH values in the lower range of normal when antithrombin was decreased  $> 75\%$  from baseline<sup>83</sup> (Figure 3C). The same observation was seen in additional cohorts of hemophilia patients with inhibitors.<sup>84</sup> Another study investigated the additional effects on TG of reducing antithrombin and coadministering either aPCC or rFVIIa. Antithrombin was reduced with an anti-antithrombin antibody to 50% and 10% of residual antithrombin. This caused an increase in TPH, which was further increased by addition of rFVIIa and aPCC. A total of 1.0 IU/ml of aPCC in plasma with 10% residual antithrombin resulted in near normalization of TG, whereas rFVIIa was not able to reach this TG level.<sup>85</sup>

## 9 | CONCLUSION AND PERSPECTIVES

The treatment of HA has evolved tremendously during recent decades. Even though FVIII replacement therapy can be monitored with FVIII assays and subsequent trough levels are determined, heterogeneity in individual bleeding phenotype still exists. The TGA can assist in profiling the individual bleeding phenotype and guide treatment based on the most important parameters (TPH and ETP). TGAs can solve the problem of the discrepant results between one-stage and chromogenic assays for monitoring recombinant and extended half-life FVIII products because it indicates the more realistic hemostatic response provided by these products compared with the assumed FVIII activity level provided by FVIII assays.<sup>86</sup> Furthermore, TGAs can be of special importance in case of break through bleeds in hemophilia patients treated with BPAs or non-factor concentrates, in which no current protocol exists to restore hemostasis safely and to guide prohemostatic therapy. In addition, when treatment modalities are used concomitantly, for example during a bleeding episode, TG effects can be monitored *in vitro* and *ex vivo* to maintain effective hemostasis and prevent safety concerns, like the combination of emicizumab and aPCC.

We have identified several essentials to be addressed before TGAs can be widely used in clinical practice to monitor treatment of HA patients:

- Comparison of different TGA platforms and performance in a variety of hyper- and hypocoagulable states, and during treatments for these conditions.



- Standardization of TGAs with uniform reporting of (local) TG reference ranges and reporting of TG parameters as percentage of normal.
- Determination of target ranges of TG profiles for treatment modalities for HA patients, comparable with trough levels of FVIII activity in patients receiving FVIII replacement therapy.
- Controlled trials to determine the clinical implication of individualized treatment (FVIII replacement therapy, BPAs, and concomitant treatments with non-factor replacement therapies) based on TG profiling and bleeding outcome in different clinical settings, such as prophylaxis, during bleeding episodes, and in the perioperative period.
- Furthermore, it would be interesting to investigate the possible role of TGA in the monitoring of gene therapy, especially in case of transaminitis.

In conclusion, without uniformly constructed clinical trials implementing TGA profiling, novel treatment modalities will face ongoing safety concerns, especially in patients with inhibitors or undergoing surgery.

#### ACKNOWLEDGMENTS

The authors thank Franka Wijnbergen for drawing Figure 3.

#### CONFLICT OF INTEREST

M.V., L.V., and S.S. do not have any conflicts of interest to declare.

#### AUTHOR CONTRIBUTIONS

Marieke J. A. Verhagen and Lars L. F. G. Valke outlined and planned the review. Marieke J. A. Verhagen performed the literature search. Marieke J. A. Verhagen and Lars L. F. G. Valke selected the articles. Marieke J. A. Verhagen wrote the first draft of the introduction, search strategy, FVIII concentrate, and BPAs. Lars L. F. G. Valke wrote the first draft of TGAs, emicizumab, anti-tissue factor pathway inhibitor, and antithrombin inhibition. Saskia E. M. Schols oversaw the execution of the review and critically revised the manuscript. All authors approved the final version of the manuscript.

#### ORCID

Marieke J. A. Verhagen  <https://orcid.org/0000-0002-0089-461X>

Lars L. F. G. Valke  <https://orcid.org/0000-0002-8638-3166>

Saskia E. M. Schols  <https://orcid.org/0000-0003-2423-2829>

#### REFERENCES

1. Srivastava A, Santagostino E, Dougall A, et al. Guidelines for the management of hemophilia. *Haemophilia*. 2020;26(Suppl 6):1-158. doi:10.1111/hae.14046
2. Iorio A. Using pharmacokinetics to individualize hemophilia therapy. *Hematology Am Soc Hematol Educ Program*. 2017;2017:595-604. doi:10.1182/asheducation-2017.1.595
3. Dargaud Y, Negrier C, Rusen L, et al. Individual thrombin generation and spontaneous bleeding rate during personalized prophylaxis with Nuwiq (human-cl rhFVIII) in previously treated patients with severe haemophilia A. *Haemophilia*. 2018;24:619-627.
4. Dargaud Y, Béguin S, Lienhart A, et al. Evaluation of thrombin generating capacity in plasma from patients with haemophilia A and B. *Thromb Haemost*. 2005;93:475-480. doi:10.1160/th04-10-0706
5. Beltrán-Miranda CP, Khan A, Jaloma-Cruz AR, Laffan MA. Thrombin generation and phenotypic correlation in haemophilia A. *Haemophilia*. 2005;11:326-334. doi:10.1111/j.1365-2516.2005.01107.x
6. Tarandovskiy ID, Balandina AN, Kopylov KG, et al. Investigation of the phenotype heterogeneity in severe hemophilia A using thromboelastography, thrombin generation, and thrombodynamics. *Thromb Res*. 2013;131:e274-e280. doi:10.1016/j.thromres.2013.04.004
7. Haghpanah S, Bazrafshan A, Silavizadeh S, Dehghani J, Afrasiabi A, Karimi M. Evaluation of thrombin generation assay in patients with hemophilia. *Clin Appl Thromb Hemost*. 2014;22:322-326.
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. doi:10.1159/000071636
9. Young G, Sørensen B, Dargaud Y, Negrier C, Brummel-Ziedins K, Key NS. Thrombin generation and whole blood viscoelastic assays in the management of hemophilia: current state of art and future perspectives. *Blood*. 2013;121:1944-1950. doi:10.1182/blood-2012-08-378935
10. Hemker HC, Giesen PL, Ramjee M, Wagenvoort R, Béguin S. The thrombogram: monitoring thrombin generation in platelet-rich plasma. *Thromb Haemost*. 2000;83:589-591.
11. Kintigh J, Monagle P, Ignjatovic V. A review of commercially available thrombin generation assays. *Res Pract Thromb Haemost*. 2018;2:42-48. doi:10.1002/rth2.12048
12. Takeyama M, Nogami K, Shima M. A new parameter in the thrombin generation assay, mean velocity to peak thrombin, reflects factor VIII activity in patients with haemophilia A. *Haemophilia*. 2016;22:e474-e477. doi:10.1111/hae.13052
13. van Geffen M, Loof A, Lap P, et al. A novel hemostasis assay for the simultaneous measurement of coagulation and fibrinolysis. *Hematology*. 2011;16:327-336. doi:10.1179/102453311x13085644680348
14. Simpson ML, Goldenberg NA, Jacobson LJ, Bombardier CG, Hathaway WE, Manco-Johnson MJ. Simultaneous thrombin and plasmin generation capacities in normal and abnormal states of coagulation and fibrinolysis in children and adults. *Thromb Res*. 2011;127:317-323. doi:10.1016/j.thromres.2010.12.011
15. Matsumoto T, Nogami K, Shima M. Simultaneous measurement of thrombin and plasmin generation to assess the interplay between coagulation and fibrinolysis. *Thromb Haemost*. 2013;110:761-768. doi:10.1160/th13-04-0345
16. Dargaud Y, Wolberg AS, Gray E, Negrier C, Hemker HC. 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. doi:10.1111/jth.13743
17. Mohammed BM, Martin EJ, Salinas V, Carmona R, Young G, Brophy DF. Failure of corn trypsin inhibitor to affect the thrombin generation assay in plasma from severe hemophiliacs. *J Thromb Haemost*. 2014;12:1558-1561. doi:10.1111/jth.12659
18. Wegert W, Harder S, Bassus S, Kirchmaier CM. Platelet-dependent thrombin generation assay for monitoring the efficacy of recombinant factor VIIa. *Platelets*. 2005;16:45-50. doi:10.1080/09537100400008059
19. van Veen JJ, Gatt A, Cooper PC, Kitchen S, Bowyer AE, Makris M. Corn trypsin inhibitor in fluorogenic thrombin-generation measurements is only necessary at low tissue factor concentrations and influences the relationship between factor VIII coagulant activity and thrombogram parameters. *Blood Coagul Fibrinolysis*. 2008;19:183-189. doi:10.1097/MBC.0b013e3282f4bb47

20. van Veen JJ, Gatt A, Bowyer AE, Cooper PC, Kitchen S, Makris M. The effect of tissue factor concentration on calibrated automated thrombography in the presence of inhibitor bypass agents. *Int J Lab Hematol*. 2009;31:189-198. doi:10.1111/j.1751-553X.2007.01022.x
21. van Veen JJ, Gatt A, Bowyer AE, Cooper PC, Kitchen S, Makris M. Calibrated automated thrombin generation and modified thromboelastometry in haemophilia A. *Thromb Res*. 2009;123:895-901. doi:10.1016/j.thromres.2008.09.011
22. Bassus S, Wegert W, Krause M, et al. Platelet-dependent coagulation assays for factor VIII efficacy measurement after substitution therapy in patients with haemophilia A. *Platelets*. 2006;17:378-384. doi:10.1080/09537100600757448
23. Mancuso ME, Fasulo MR. Thrombin generation assay as a laboratory monitoring tool during bypassing therapy in patients with hemophilia and inhibitors. *Semin Thromb Hemost*. 2016;42:30-35. doi:10.1055/s-0035-1564842
24. Lewis SJ, Stephens E, Florou G, et al. Measurement of global haemostasis in severe haemophilia A following factor VIII infusion. *Br J Haematol*. 2007;138:775-782. doi:10.1111/j.1365-2141.2007.06722.x
25. Ay Y, Balkan C, Karapinar DY, Akin M, Bilenoğlu B, Kavaklı K. Feasibility of using thrombin generation assay (TGA) for monitoring of haemostasis during supplementation therapy in haemophilic patients without inhibitors. *Haemophilia*. 2012;18:911-916. doi:10.1111/j.1365-2516.2012.02849.x
26. Al Hawaj MA, Martin EJ, Venitz J, et al. Monitoring rFVIII prophylaxis dosing using global haemostasis assays. *Haemophilia*. 2013;19:409-414. doi:10.1111/hae.12110
27. Valke L, Bukkems LH, Barteling W, et al. Pharmacodynamic monitoring of factor VIII replacement therapy in hemophilia A: combining thrombin and plasmin generation. *J Thromb Haemost*. 2020;18:3222-3231. doi:10.1111/jth.15106
28. Augustsson C, Norström E, Lind V, Martin M, Astermark J, Strandberg K. Validation of factor VIII activity for monitoring standard and extended half-life products and correlation to thrombin generation assays. *Haemophilia*. 2021;27(3):494-500. doi:10.1111/hae.14317
29. Hugenholtz GC, Luddington R, Baglin T. Haemostatic response to factor VIII administration in patients with haemophilia A measured by thrombin generation and correlation with factor concentrate use. *Haemophilia*. 2016;22:e42-e45. doi:10.1111/hae.12798
30. Delavenne X, Ollier E, Lienhart A, Dargaud Y. A new paradigm for personalized prophylaxis for patients with severe haemophilia A. *Haemophilia*. 2020;26:228-235. doi:10.1111/hae.13935
31. Brophy DF, Martin EJ, Ninivaggi M, et al. Evaluating the thrombin generation profiles of four different rFVIII products in FVIII-deficient plasma using FIXa and FXIa activation. *Haemophilia*. 2018;24:815-822. doi:10.1111/hae.13597
32. Salvagno GL, Astermark J, Lippi G, et al. Thrombin generation assay: a useful routine check-up tool in the management of patients with haemophilia? *Haemophilia*. 2009;15:290-296. doi:10.1111/j.1365-2516.2008.01877.x
33. Chelle P, Montmartin A, Piot M, et al. Prediction of individual factor VIII or IX level for the correction of thrombin generation in haemophilic patients. *Haemophilia*. 2018;24:995-1001. doi:10.1111/hae.13539
34. Dargaud Y, Bordet JC, Lienhart A, Negrier C. Use of the thrombin generation test to evaluate response to treatment with recombinant activated factor VII. *Semin Hematol*. 2008;45:S72-S73. doi:10.1053/j.seminhematol.2008.03.008
35. Varadi K, Negrier C, Berntorp E, et al. Monitoring the bioavailability of FEIBA with a thrombin generation assay. *J Thromb Haemost*. 2003;1:2374-2380. doi:10.1046/j.1538-7836.2003.00450.x
36. Luna-Záizar H, Beltrán-Miranda CP, Esparza-Flores MA, et al. Thrombin generation as objective parameter of treatment response in patients with severe haemophilia A and high-titre inhibitors. *Haemophilia*. 2014;20:e7-e14. doi:10.1111/hae.12309
37. Ettingshausen CE, Kreuz W. Early long-term FEIBA prophylaxis in haemophilia A patients with inhibitor after failing immune tolerance induction: a prospective clinical case series. *Haemophilia*. 2010;16:90-100. doi:10.1111/j.1365-2516.2009.02116.x
38. Eichinger S, Lubczyk B, Kollars M, et al. Thrombin generation in haemophilia A patients with factor VIII inhibitors after infusion of recombinant factor VIIa. *Eur J Clin Invest*. 2009;39:707-713. doi:10.1111/j.1365-2362.2009.02168.x
39. Fernández-Bello I, Stenmo C, Butta N, Lind V, Ezban M, Jiménez-Yuste V. The pharmacokinetics and pharmacodynamics of single-dose and multiple-dose recombinant activated factor VII in patients with haemophilia A or B. *Haemophilia*. 2017;23:868-876. doi:10.1111/hae.13312
40. Qi X, Zhao Y, Li K, Fan L, Hua B. Evaluating and monitoring the efficacy of recombinant activated factor VIIa in patients with haemophilia and inhibitors. *Blood Coagul Fibrinolysis*. 2014;25:754-760. doi:10.1097/mbc.0000000000000137
41. Tran HT, Sørensen B, Bjørnsen S, Pripp AH, Tjønnfjord GE, Andre HP. Monitoring bypassing agent therapy - a prospective crossover study comparing thromboelastometry and thrombin generation assay. *Haemophilia*. 2015;21:275-283. doi:10.1111/hae.12570
42. Dargaud Y, Lienhart A, Negrier C. Prospective assessment of thrombin generation test for dose monitoring of bypassing therapy in hemophilia patients with inhibitors undergoing elective surgery. *Blood*. 2010;116:5734-5737. doi:10.1182/blood-2010-06-291906
43. Mancuso ME, Chantarangkul V, Clerici M, et al. Low thrombin generation during major orthopaedic surgery fails to predict the bleeding risk in inhibitor patients treated with bypassing agents. *Haemophilia*. 2016;22:e292-e300. doi:10.1111/hae.12939
44. Spira J, Plyushch O, Zozulya N, et al. Safety, pharmacokinetics and efficacy of factor VIIa formulated with PEGylated liposomes in haemophilia A patients with inhibitors to factor VIII - an open label, exploratory, cross-over, phase I/II study. *Haemophilia*. 2010;16:910-918.
45. Shirahata A, Fukutake K, Mimaya J, et al. Results of clot waveform analysis and thrombin generation test for a plasma-derived factor VIIa and X mixture (MC710) in haemophilia patients with inhibitors-phase I trial: 2nd report. *Haemophilia*. 2013;19:330-337.
46. Gruppo RA, Malan D, Kapocsi J, et al. Phase 1, single-dose escalating study of marzeptacog alfa (activated), a recombinant factor VIIa variant, in patients with severe hemophilia. *J Thromb Haemost*. 2018;16:1984-1993. doi:10.1111/jth.14247
47. Bar-Ilan A, Livnat T, Hoffmann M, et al. In vitro characterization of MOD-5014, a novel long-acting carboxy-terminal peptide (CTP)-modified activated FVII. *Haemophilia*. 2018;24:477-486. doi:10.1111/hae.13428
48. Uchida N, Sambe T, Yoneyama K, et al. A first-in-human phase 1 study of ACE910, a novel factor VIII-mimetic bispecific antibody, in healthy subjects. *Blood*. 2016;127:1633-1641. doi:10.1182/blood-2015-06-650226
49. Hartmann R, Feenstra T, Knappe S, Schrenk G, Scheiflinger F, Dockal M. A bispecific antibody demonstrates limited measurability in routine coagulation assays. *Blood Coagul Fibrinolysis*. 2020;31:353-365. doi:10.1097/mbc.0000000000000921
50. Oldenburg J, Mahlangu JN, Kim B, et al. Efficacy of emicizumab prophylaxis in hemophilia A with inhibitors. *N Engl J Med*. 2017;377:809-818. doi:10.1056/NEJMoa1703068
51. Schmitt C, Adamkewicz JI, Xu J, et al. Pharmacokinetics and pharmacodynamics of emicizumab in persons with hemophilia A with factor VIII inhibitors: HAVEN 1 study. *Thromb Haemost*. 2021;121:351-360. doi:10.1055/s-0040-1717114
52. Kizilocak H, Marquez-Casas E, Malvar J, Carmona R, Young G. Determining the approximate factor VIII level of patients with

- severe haemophilia A on emicizumab using in vivo global haemostasis assays. *Haemophilia*. 2021;27:730-735. doi:10.1111/hae.14359
53. Barg AA, Budnik I, Avishai E, et al. Emicizumab prophylaxis: prospective longitudinal real-world follow-up and monitoring. *Haemophilia*. 2021;27(3):383-391. doi:10.1111/hae.14318
  54. Misgav M, Brutman-Barazani T, Budnik I, et al. Emicizumab prophylaxis in haemophilia patients older than 50 years with cardiovascular risk factors: real-world data. *Haemophilia*. 2021;27:253-260. doi:10.1111/hae.14261
  55. Barg AA, Livnat T, Budnik I, et al. Emicizumab treatment and monitoring in a paediatric cohort: real-world data. *Br J Haematol*. 2020;191:282-290. doi:10.1111/bjh.16964
  56. Barg AA, Avishai E, Budnik I, et al. Emicizumab prophylaxis among infants and toddlers with severe hemophilia A and inhibitors-a single-center cohort. *Pediatr Blood Cancer*. 2019;66:e27886. doi:10.1002/pbc.27886
  57. Brophy DF, Martin EJ, Kuhn J. Use of global assays to monitor emicizumab prophylactic therapy in patients with haemophilia A with inhibitors. *Haemophilia*. 2019;25:e121-e123. doi:10.1111/hae.13689
  58. Okamoto S, Suzuki N, Suzuki A, et al. Successful perioperative combination of high-dose FVIII therapy followed by emicizumab in a patient with hemophilia A with inhibitors. *TH Open*. 2019;3:e364-e366. doi:10.1055/s-0039-3401001
  59. Teo HKW, Wong WH, Lam JCM. Recurrent intracranial bleed in a child receiving prophylaxis with emicizumab. *Haemophilia*. 2021;27:e415-e418. doi:10.1111/hae.14274
  60. Bravo MI, Raventós A, Pérez A, Costa M, Willis T. Non-additive effect on thrombin generation when a plasma-derived factor VIII/von Willebrand factor (FVIII/VWF) is combined with emicizumab in vitro. *J Thromb Haemost*. 2020;18:1934-1939. doi:10.1111/jth.14887
  61. Dargaud Y, Lienhart A, Janbain M, Le Quellec S, Enjolras N, Negrier C. Use of thrombin generation assay to personalize treatment of breakthrough bleeds in a patient with hemophilia and inhibitors receiving prophylaxis with emicizumab. *Haematologica*. 2018;103:e181-e183. doi:10.3324/haematol.2017.185330
  62. Kizilocak H, Yukhtman CL, Marquez-Casas E, Lee J, Donkin J, Young G. Management of perioperative hemostasis in a severe hemophilia A patient with inhibitors on emicizumab using global hemostasis assays. *Ther Adv Hematol*. 2019;10:2040620719860025. doi:10.1177/2040620719860025
  63. Valke LFFG, Beckers EAM, Blijlevens NMA, van Heerde WL, Schols SEM. Effect of emicizumab on thrombin generation: a case report of breakthrough bleeding during emicizumab treatment. *Haemophilia*. 2020;26:e327-e330.
  64. Lockhart M, Tardy-Poncet B, Montmartin A, Noyel P, Thouvenin S, Berger C. Surgery with emicizumab prophylaxis for two paediatric patients with severe haemophilia A with inhibitors. *Pediatr Blood Cancer*. 2021;68:e29041. doi:10.1002/pbc.29041
  65. Mizumachi K, Tsumura Y, Nakajima Y, Koh K, Nogami K. Clot waveform analysis for perioperative hemostatic monitoring of arthroscopic synovectomy in a pediatric patient with hemophilia A and inhibitor receiving emicizumab prophylaxis. *Int J Hematol*. 2021;113(6):930-935. doi:10.1007/s12185-021-03095-w
  66. Santagostino E, Mancuso ME, Novembrino C, Solimeno LP, Tripodi A, Peyvandi F. Rescue factor VIII replacement to secure hemostasis in a patient with hemophilia A and inhibitors on emicizumab prophylaxis undergoing hip replacement. *Haematologica*. 2019;104:e380-e382. doi:10.3324/haematol.2018.215129
  67. Kizilocak H, Marquez-Casas E, Phei Wee C, Malvar J, Carmona R, Young G. Comparison of bypassing agents in patients on emicizumab using global hemostasis assays. *Haemophilia*. 2021;27:164-172. doi:10.1111/hae.14213
  68. Schultz NH, Glosli H, Bjørnsen S, Holme PA. The effect of emicizumab and bypassing agents in patients with hemophilia - an in vitro study. *Res Pract Thromb Haemost*. 2021;5:e12561. doi:10.1002/rth2.12561
  69. Grandoni J, Duret V, Bonzo D, Evans S, Plantier JL. Exploratory in vitro evaluation of thrombin generation of eptacog beta (recombinant human FVIIa) and emicizumab in congenital haemophilia A plasma. *Haemophilia*. 2021;27:321-328. doi:10.1111/hae.14253
  70. Hartmann R, Feenstra T, Valentino L, Dockal M, Scheiflinger F. In vitro studies show synergistic effects of a procoagulant bispecific antibody and bypassing agents. *J Thromb Haemost*. 2018;16:1580-1591. doi:10.1111/jth.14203
  71. Müller J, Pekrul I, Pöttsch B, Berning B, Oldenburg J, Spannagl M. Laboratory monitoring in emicizumab-treated persons with hemophilia A. *Thromb Haemost*. 2019;119:1384-1393. doi:10.1055/s-0039-1692427
  72. Nordfang O, Valentin S, Beck TC, Hedner U. Inhibition of extrinsic pathway inhibitor shortens the coagulation time of normal plasma and of hemophilia plasma. *Thromb Haemost*. 1991;66:464-467.
  73. Ellery PER, Adams MJ. *Tissue factor pathway inhibitor: then and now*. Semin Thromb Hemostasis 2015: 123.
  74. Kjalke M, Kjelgaard-Hansen M, Andersen S, Hilden I. Thrombin generation potential in the presence of concizumab and rFVIIa, aPCC, rFVIII or rFIX: In vitro and ex vivo analyses. *J Thromb Haemost*. 2021;19:1687-1696. doi:10.1111/jth.15323
  75. Gorczyca ME, Nair SC, Jilma B, et al. Inhibition of tissue factor pathway inhibitor by the aptamer BAX499 improves clotting of hemophilic blood and plasma. *J Thromb Haemost*. 2012;10:1581-1590. doi:10.1111/j.1538-7836.2012.04790.x
  76. Dockal M, Pachlinger R, Hartmann R, et al. Biological explanation of clinically observed elevation of TFPI plasma levels after treatment with TFPI-antagonistic Aptamer BAX 499. *Blood*. 2012;120:1104.
  77. Patel-Hett S, Martin EJ, Mohammed BM, et al. Marstacimab, a tissue factor pathway inhibitor neutralizing antibody, improves coagulation parameters of ex vivo dosed haemophilic blood and plasmas. *Haemophilia*. 2019;25:797-806. doi:10.1111/hae.13820
  78. Martin EJ, Nolte ME, Kuhn J, Schmidt N, Pfaff N, Brophy DF. An in vitro pharmacodynamic spiking study of befovacimab, a tissue factor pathway inhibitor monoclonal antibody, in blood samples from patients with severe FVIII deficiency. *Haemophilia*. 2021;27:690-698. doi:10.1111/hae.14314
  79. Waters EK, Sigh J, Friedrich U, Hilden I, Sørensen BB. Concizumab, an anti-tissue factor pathway inhibitor antibody, induces increased thrombin generation in plasma from haemophilia patients and healthy subjects measured by the thrombin generation assay. *Haemophilia*. 2017;23:769-776. doi:10.1111/hae.13260
  80. Eichler H, Angchaisuksiri P, Kavakli K, et al. A randomized trial of safety, pharmacokinetics and pharmacodynamics of concizumab in people with hemophilia A. *J Thromb Haemost*. 2018;16:2184-2195. doi:10.1111/jth.14272
  81. Eichler H, Angchaisuksiri P, Kavakli K, et al. Concizumab restores thrombin generation potential in patients with haemophilia: pharmacokinetic/pharmacodynamic modelling results of concizumab phase 1/1b data. *Haemophilia*. 2019;25:60-66. doi:10.1111/hae.13627
  82. Shapiro AD, Angchaisuksiri P, Astermark J, et al. Subcutaneous concizumab prophylaxis in hemophilia A and hemophilia A/B with inhibitors: phase 2 trial results. *Blood*. 2019;134:1973-1982. doi:10.1182/blood.2019001542
  83. Pasi KJ, Rangarajan S, Georgiev P, et al. Targeting of antithrombin in hemophilia A or B with RNAi therapy. *N Engl J Med*. 2017;377:819-828. doi:10.1056/NEJMoa1616569
  84. Pasi KJ, Lissitchkov T, Mamonov V, et al. Targeting of antithrombin in hemophilia A or B with investigational siRNA therapeutic fitusiran - results of the phase 1 inhibitor cohort. *J Thromb Haemost*. 2021;19(6):1436-1446. doi:10.1111/jth.15270
  85. Livnat T, Sehgal A, Qian K, et al. Thrombin generation in plasma of patients with haemophilia A and B with inhibitors: effects of bypassing agents and antithrombin reduction. *Blood Cells Mol Dis*. 2020;82:102416. doi:10.1016/j.bcmd.2020.102416

86. Müller J, Goldmann G, Marquardt N, Pötzsch B, Oldenburg J. Extended half-life factor VIII/factor IX products: assay discrepancies and implications for hemophilia management. *Hamostaseologie*. 2020;40:S15-s20. doi:10.1055/a-1282-2251

#### SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

**How to cite this article:** Verhagen MJA, Valke LLFG, Schols SEM. Thrombin generation for monitoring hemostatic therapy in hemophilia A: A narrative review. *J Thromb Haemost*. 2022;20:794–805. doi:[10.1111/jth.15640](https://doi.org/10.1111/jth.15640)