Individualization of bypassing agent treatment for haemophilic patients with inhibitors utilizing thromboelastography

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Summary. The treatment of bleeding for haemophilic patients with inhibitors relies on the use of the bypassing agents, recombinant factor VIIa and factor eight inhibitor bypass activity (FEIBA). While both therapies are effective in the majority of bleeding episodes, there is a significant amount of interindividual variability when it comes to the response to therapy. As of yet, there is no reliable laboratory parameter that can predict the response to therapy in the same manner that factor VIII and factor IX levels predict response in non-inhibitor patients. Developing such a laboratory parameter is vital in order to maximize the clinical efficacy of these agents. Thromboelastography (TEG) is a device, which assesses clot formation over time in whole blood and has several characteristics which suggest it may be an effective way to monitor bypass agent therapy. We studied the ability of TEG to individualize the treatment regimens of three patients with high titre inhibitors assessing the response to recombinant factor VIIa, FEIBA, and when both were used sequentially. The TEG allowed for individualization of treatment for each of the three patients and resulted in more effective, convenient and less expensive treatment regimens. We thus believe that TEG is a promising device for monitoring of bypass agent therapy and should be studied further.

Keywords: haemophilia, inhibitors, thromboelastography, treatment

Introduction

Haemophilia results from the deficiency of either factor VIII (FVIII) or factor IX (FIX) leading to a lifelong bleeding disorder with frequent bleeding into deep tissues such as the joints and muscles. With the advent of replacement therapy in the form of purified (and now recombinant) FVIII and FIX, patients can lead nearly normal lives with few bleeding episodes especially if they are receiving factor prophylactically. Unfortunately, approximately 15% of patients develop neutralizing antibodies (inhibitors) against these therapies rendering them ineffective [1]. While the inhibitor may be eradicated via immune tolerance therapy, so-called inhibitor patients often develop serious bleeding episodes prior to and during immune tolerance and approximately 30% of patients with inhibitors fail to respond to immune tolerance and have inhibitors for life.

Treating bleeding episodes for inhibitor patients requires what has come to be known as bypass therapy. Currently, there are two bypass agents available, recombinant activated factor VII (rFVIIa, Novoseven; Novo Nordisk, Bagsvaerd, Denmark) and factor eight inhibitor bypass activity [FEIBA; Baxter, Glendale, CA, USA] [2]. Porcine FVIII, another bypass agent is no longer available though a recombinant form is in development [3]. These agents are not as effective as native FVIII or FIX in controlling bleeding and importantly, there is no accepted method to monitor the clinical effectiveness. While for non-inhibitor patients, measurement of the level of the specific factor is correlated with the expected clinical response, there is no laboratory assay that predicts the clinical response of bypass agents. This becomes even more important as there appears to be significant interindividual differences in how patients respond to bypass therapy which becomes critically important during surgical procedures [2].

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Several laboratory assays have been proposed to be potentially useful for the monitoring of bypass therapy [4]. These include the activated clotting time, thrombin generation test, thromboelastography (TEG), activated partial thromboplastin time (aPTT) waveform analysis, and others. There are specific advantages and disadvantages to each method. The ideal assay would be one that predicts the clinical response, is simple, inexpensive and easy to use and gives rapid and reproducible results. In addition, portability would be a great benefit such that it can be used in the patient-care setting (operating room, clinic, etc.). Finally, a system which can utilize whole blood is desired as it may better reflect global haemostasis and would require less laboratory handling of the patient sample. One assay which fits these criteria is the TEG. In this report, we describe the use of TEG to individualize treatment regimens for haemophilia patients with inhibitors receiving bypass therapy.

Patients and methods

Three haemophilic patients with high-titre inhibitors (two FVIII and one FIX) were asked to participate in a study designed to assess their response to rFVIIa and FEIBA with the express goal of tailoring their treatment regimen to the agent(s) and schedule that generated the best clot formation as assessed by TEG. All three patients had failed multiple attempts at immune tolerance therapy. The patients/guardians gave their informed consent to participate. All data regarding bleeds, factor use, response to treatment, and hospitalizations were retrieved from the inpatient and outpatient medical records, patient diaries and pharmacy records.

We utilized the TEG® 5000 Thromboelastograph® Hemostasis Analyzer (Haemoscope, Niles, IL, USA) with TEG® Analytical Software Version 3 (initially) and 4 (Haemoscope). The upgraded software version in no way affects test results. All the assays were activated by kaolin per the manufacturers’ instructions. Activation with kaolin results in reduced interassay variability and an approximate 50% reduction in assay time. This is similar to the principle of the aPTT when compared with the older partial thromboplastin time. The kaolin was purchased from Haemoscope and consists of kaolin, buffered stabilizers and a blend of phospholipids. Of note, other studies have utilized tissue factor as the activator (see Discussion) and a trial comparing kaolin to tissue factor as an activating agent for TEG is under way.

Patients were brought to the outpatient clinic in the non-bleeding state (no active bleeds and no factor for the previous 48 h). An initial TEG tracing was performed followed by treatment with rFVIIa, FEIBA or both sequentially (during multiple visits) and serial blood samples were drawn. The samples were all drawn from central venous access devices after a discard of at least 5 mL. The native (non-citrated) blood samples were activated with kaolin and placed into the device within 4 min of sampling at 37°C. A tracing and multiple quantitative parameters were captured including R which measures clot initiation, K and angle which measure clot propagation, MA which measures peak clot rigidity and G which measures clot elastic modulus.

Patient 1 was 9 years old at the time of assessment and has a high-titre inhibitor ranging from 60 to 300 BU with a historical peak titre of >300 000 BU. According to his mother, he had a poor response to rFVIIa even at doses of >200 µg kg⁻¹ but did respond well to FEIBA 75 units kg⁻¹. He has severe and recurrent haemarthroses and is wheelchair bound. Patient 2 is 17 years old with FVIII deficiency with an inhibitor titre ranging from 40 to 120 with a peak historical titre of >600 BU. He also has recurrent, severe haemarthroses and several years before this trial underwent an above the knee amputation due to a severe pseudotumour which led to gangrene secondary to destruction of the arteries of the leg. He is also wheelchair bound. As per patient report, he responds well to both rFVIIa and FEIBA and generally uses rFVIIa as first line replacing it with FEIBA if the bleed does not resolve. The third patient is 16 years old with FIX deficiency and also with an inhibitor titre in the 70–100 range. He has end-stage arthropathy of one knee and one shoulder. He had been wheelchair bound until he had undergone a total knee arthroplasty. As per his report, he responds well to both rFVIIa and FEIBA.

Depending on each patient’s response, treatment regimens were developed to best manage bleeding episodes. Although testing was performed in the non-bleeding state, we elected to use those results to plan treatment regimens for active bleeding. We then compared their previous response to treatment with the new regimen. As the sample size is small, detailed statistical analysis could not be performed and thus only descriptive statistics are used.

Results

Patient 1

After obtaining a pretreatment TEG tracing, this patient received rFVIIa 200 µg kg⁻¹, and the tracing prior to and 15 min following rFVIIa was flat.
indicating no clot formation (Fig. 1) consistent with the mother’s report of a poor response. On the contrary this patient had an excellent clinical and TEG response to FEIBA (Fig. 2 and Table 1). A TEG pharmacokinetic analysis was performed after this single dose of FEIBA demonstrating a gradual decay in clot formation over time. As per our practice of treating severe bleeds with sequential combination therapy [5] (FEIBA followed by rFVIIa or vice versa), we performed the same experiment as above with FEIBA only this time we gave a dose of rFVIIa 200 µg kg⁻¹ at the 4-h time point (Fig. 3 and Table 2). Interestingly, this patient who previously had no response to rFVIIa now demonstrated a clear improvement in TEG parameters when rFVIIa was given while there was still FEIBA activity as measured by the TEG. Additional experiments adding the rFVIIa at other time points demonstrated that a dose given 2 h after the FEIBA dose gave the best clot tracing (not shown).

These experiments culminated in a new regimen for this patient. At the onset of bleeding, he receives FEIBA 75 units kg⁻¹ which the family may re-dose after 8 h (based on the significant decay noted at that time point). If his bleed does not respond then he is administered rFVIIa 200 µg kg⁻¹ 2 or 4 h after the FEIBA dose. This regimen has been used effectively for nearly 2 years, and although it is not 100% effective, it is far more effective than his previous regimen which essentially did not use rFVIIa at all. Importantly, this regimen has resulted in more rapid resolution of bleeds, fewer hospitalizations and an improved quality of life as a result.

**Patient 2**

After obtaining a baseline tracing, this patient underwent two separate pharmacokinetic studies, one with FEIBA and the other with rFVIIa. He received FEIBA 75 units kg⁻¹ and rFVIIa 180 µg kg⁻¹. This patient who clinically responds well to both FEIBA and rFVIIa demonstrated a significantly improved clot curve and TEG parameters with both rFVIIa and FEIBA. His tracings also demonstrated an expected decay in the curves over time from infusion. Importantly, the rFVIIa curve

![Fig. 1. Thromboelastography tracings for patient 1 before and 15 min after recombinant activated factor VII 200 µg kg⁻¹.](image1)

![Fig. 2. Thromboelastography tracings for patient 1 before and after factor eight inhibitor bypass activity 75 units kg⁻¹.](image2)

**Table 1.** Thromboelastography parameters of patient 1 before and after factor eight inhibitor bypass activity (FEIBA) 75 units kg⁻¹.

<table>
<thead>
<tr>
<th></th>
<th>R (min)</th>
<th>K (min)</th>
<th>Angle (degrees)</th>
<th>MA (mm)</th>
<th>G (dynes cm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal range</td>
<td>4–8</td>
<td>1–4</td>
<td>47–74</td>
<td>55–73</td>
<td>6–13.2</td>
</tr>
<tr>
<td>Pre-FEIBA</td>
<td>101.2</td>
<td>34</td>
<td>7.2</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>Post-FEIBA</td>
<td>23</td>
<td>3.1</td>
<td>51.8</td>
<td>63.6</td>
<td>8.8</td>
</tr>
</tbody>
</table>

NM, not measurable.

![Fig. 3. Thromboelastography tracings for patient 1 before and after factor eight inhibitor bypass activity 75 units kg⁻¹ followed by recombinant activated factor VII 200 µg kg⁻¹.](image3)

**Table 2.** Thromboelastography parameters of patient 1 after factor eight inhibitor bypass activity (FEIBA) followed by recombinant activated FVII.

<table>
<thead>
<tr>
<th></th>
<th>R (min)</th>
<th>K (min)</th>
<th>Angle (degrees)</th>
<th>MA (mm)</th>
<th>G (dynes cm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal range</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-FEIBA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 min post-FEIBA</td>
<td>12.4</td>
<td>3.4</td>
<td>48.7</td>
<td>65.4</td>
<td>9.5</td>
</tr>
<tr>
<td>4 h post-FEIBA</td>
<td>34.5</td>
<td>6.7</td>
<td>32.7</td>
<td>65.3</td>
<td>9.4</td>
</tr>
<tr>
<td>15 min post-rFVIIa (4:15 post-FEIBA)</td>
<td>10.3</td>
<td>2.8</td>
<td>53.7</td>
<td>64.2</td>
<td>9</td>
</tr>
</tbody>
</table>

NM, not measurable.
still demonstrated an improved (over baseline) clot formation even at the 6-h time point (Fig. 4 and Table 3) suggesting that at this dose of rFVIIa, dosing can be given every 6 h rather than every 2 h. In particular, clot rigidity (MA) and clot elasticity (G parameter) remained in the normal range even at this time point (Table 3). There was an equally good clot formation curve following FEIBA (as in patient 1) with a decay similar to although not as rapid as with rFVIIa. While this did not lead to a change in the way he utilizes FEIBA, the TEG result concurred with the patient’s clinical response.

In addition to the studies with rFVIIa and FEIBA alone, this patient also underwent a study similar to patient 1 whereby both rFVIIa and FEIBA were utilized in a sequential fashion; however, in this patient rFVIIa was given first. These results mirror those of patient 3 whose data are presented below. As a result of these experiments in patient 2, his treatment regimen has been altered such that he only doses rFVIIa every 6 h rather than every 2–3 h with no loss of efficacy resulting in a more than 50% reduction in factor usage. In addition, this has improved his quality of life during bleeding episodes by reducing the number of infusions required to treat a bleed. Furthermore, for more problematic bleeds (ones for which he is hospitalized), he is treated with an alternating regimen of rFVIIa and FEIBA given every 6 h as per our previous report [5].

Patient 3

The experiments performed with patient 3 were the same as those for patient 2. The sequential rFVIIa followed by FEIBA experiment was conducted as follows. After obtaining a baseline tracing, a dose of rFVIIa 180 µg kg⁻¹ was given and serial TEG tracings were carried out at 15 min, 2, 4 and 6 h. At hour 6, FEIBA 75 units kg⁻¹ was given. The TEG tracings after rFVIIa were similar to those obtained previously in this patient (data not shown) with a decay over time. Fifteen minutes after receiving the dose of FEIBA, another tracing was performed. Interestingly, this tracing gave the best clot initiation (R) and propagation (K and angle) even better than immediately after the rFVIIa alone; (Fig. 5 and Table 4). Additional experiments confirmed these findings and demonstrated that a dose of FEIBA 4–6 h after rFVIIa always resulted in a clot formation curve which was normal. He is now treated in a manner similar to patient 2 both for outpatient and inpatient management. His factor usage has decreased by more than 50% as a result of less frequent dosing of rFVIIa and his response to severe bleeds with sequential therapy has been maximized based on the results of the TEG tracings for those experiments. This has led to a reduction in hospitalizations by 33% as well as reduced lengths of stay (from a median of 7 days to a median of 3 days) when hospitalized for bleeds.

Discussion

The clinical response to bypass therapy (rFVIIa and FEIBA) has significant interindividual variability for reasons that are currently unclear [2]. Our patients also demonstrate this variability (Tables 5 and 6). Presently, the only way to determine a patient’s response is by trial and error as no assay has yet demonstrated the ability to predict the clinical response to bypassing agents. Furthermore, the selection of a dosing regimen is based on the results of clinical trials, however, applying this data to each individual patient is problematic. What is needed is an assay that can predict the clinical response and allow for the individualization of bypass therapy, which can result in not only better outcomes but potentially better quality of life and reduced cost.

Several devices and assays are being evaluated for this purpose [4]. The major advantages of TEG are that it provides reliable and reproducible results [6], is easy to use and portable, is relatively inexpensive, and provides results rapidly. Finally, the assay utilizes whole blood, which arguably is the best tissue for which to evaluate the coagulation process.

![Fig. 4. Thromboelastography tracings for patient 2 before and after recombinant activated factor VII 180 µg kg⁻¹.](image)

| Table 3. Thromboelastography parameters of patient 2 before and after recombinant activated factor VII (rFVIIa). |
|---|---|---|---|---|---|
| R (min) | K (min) | Angle (degrees) | MA (mm) | G (dynes cm⁻²) |
| Normal range | 4–8 | 1–4 | 47–74 | 55–73 | 6–13.2 |
| Pre-rFVIIa | 91.6 | 82 | NM | NM | NM |
| 15 min post-rFVIIa | 5.4 | 2.3 | 52.3 | 76.6 | 16.4 |
| 6 h post-rFVIIa | 57.8 | 21.2 | 11.7 | 68.8 | 11 |

NM, not measurable.
Prior experience with TEG utilizing the ROTEG/ROTEM (Pentapharm, Munich, Germany) which functionally is identical to the TEG using ex vivo spiking of samples has demonstrated the utility of this instrument in the assessment of various aspects of haemophilia. The pioneering studies by Sorensen and co-workers [7–9] have shown that TEG can distinguish patients with mild, moderate and severe haemophilia as well as demonstrate improvement in clot characteristics after ex vivo addition of FVIII, FIX and bypassing agents. These excellent studies have laid the foundation for developing TEG as a monitoring device for patients with haemophilia and inhibitors receiving bypassing agents.

The results for patient 1 (rFVIIa non-responder) demonstrated clearly that rFVIIa (at least alone) did not improve his clot formation curves. The reasons for this are not completely clear although we were able to demonstrate an antibody to rFVIIa immuno-logically although we cannot state based on this assay whether this antibody neutralized the function of rFVIIa [10]. While, FEIBA contains FVIIa, his response probably relates to the presence of the other components, namely activated factor X (FX) and factor II. For patients 2 and 3 the TEG studies simply pointed out that clot formation remains excellent for 6 h after a dose of rFVIIa. All three patients respond well to FEIBA and all three demonstrated an excellent response as measured by the TEG parameters indicating that TEG is predictive of the clinical response to FEIBA as well.

We first reported the use of sequential therapy with FEIBA and rFVIIa in individuals with particularly severe bleeds 2 years ago, however, did not at the time have TEG data to report [5]. As a result of these experiments, all three patients use sequential therapy if they fail to respond to rFVIIa or FEIBA alone and...
we have been able to maximize outcome and minimize factor usage by utilizing the regimen, which results in the best clot characteristics. We speculate that the additive effect of these agents as demonstrated by TEG is likely due to the presence of pharmacological amounts of FVIIa (from rFVIIa) along with increased amounts of FX and prothrombin, the major prothrombotic components of FEIBA. Laboratory studies have suggested that rFVIIa is more effective in the presence of increasing amounts of FX and prothrombin supporting the notion that rFVIIa and FEIBA given sequentially (or perhaps together) would have additive if not synergistic effects [11].

There are several limitations to the present study. Our patient experiments were conducted in the non-bleeding state and although one may presume the pharmacokinetics during the bleeding state will be different, when we applied the results to the bleeding state, the efficacy seemed to remain. The lack of a control group and lack of blinding of the patients and treaters allows for the introduction of bias as to the individual patient outcomes. The small numbers of patients in our study may not allow our results to be generalized to all inhibitor patients. As, we used kaolin to activate clot formation which activates the intrinsic system, the clot formation in our samples may not be as physiologic had we used tissue factor; however, as each patient served as their own control (their own baseline curve prior to adding bypassing agents), this should not affect outcome. Lastly, patient samples were obtained from central venous catheters which are instilled with heparin when not in use, and although the first 5 mL of each sample was discarded, residual heparin may have remained in the sample and affected the TEG results. This potential problem, however, was overcome by each patient serving as his own control and the results for multiple baseline samples for each patient were consistent including the patient with no response to rFVIIa. Additional studies are under way to determine the ideal analytic conditions for TEG which include a comparison of kaolin and tissue factor as activating agents.

In summary, we have demonstrated the utility of TEG for the individualization of bypass therapy in haemophilic patients with bleeding episodes. This approach allowed for maximizing patient outcomes while reducing factor consumption. This led to improved management of bleeding episodes with more rapid resolution of bleeds by 2–3 days on average and a reduction of hospitalizations by 33–50% for the three patients. This consequently resulted in improved quality of life by reducing hospitalizations, more rapid resolution of bleeds and fewer required infusions with each bleed. Finally, individualizing therapy led to a reduction in cost by reducing the amount of factor used by approximately 50% for the three patients in aggregate and reducing hospitalizations. Although a formal pharmacoeconomic analysis was not performed, the cost for the outpatient visits and performing TEG (about $5 per assay) was negligible when compared with the cost of factor.

Further studies, including larger, well-designed prospective studies are needed to confirm our findings before we can recommend this approach to all inhibitor patients. These studies are currently under way and will hopefully lead to improved management for inhibitor patients receiving bypassing agents.

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