Brief Report

Prospective assessment of thrombin generation test for dose monitoring of bypassing therapy in hemophilia patients with inhibitors undergoing elective surgery

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ABSTRACT

Clinical response to bypassing agents (BPA) may vary between patients. Surgery is a particular situation which requires effective hemostasis during the procedure and for several days postoperatively, in order to obtain satisfactory wound healing. However, the optimal dose of BPA in different surgical situations has not been clearly established. We report here a prospective assessment of thrombin generation test (TGT) in monitoring the effectiveness of BPA during 10 elective invasive procedures performed in 6 patients with severe hemophilia and high-titre inhibitors. A standardized 3 step-protocol was used in all cases to individually tailor BPA. Thrombin generating capacity of patients increased after in-vitro and ex-vivo addition of BPA in a dose-dependent manner. Our results also showed a correlation between in-vivo clinical response to BPA and thrombin generating capacity. These data suggests that TGT may represent a surrogate marker for monitoring bypassing therapies in surgical situations.

Key words: Hemophilia, inhibitors, recombinant factor VIIa, APCC, thrombin generation assay
INTRODUCTION

The development of inhibitors is one of the most serious complications of hemophilia and bleeding episodes are more difficult to control in these patients(1). Several studies have reported excellent efficacy of FEIBA® (Baxter, Vienna, Austria) and NovoSeven® (Novo Nordisk, Copenhagen, Denmark) in 80 to 85% of cases(2-4). Nevertheless, the presence of an inhibitor demands extremely cautious surgical management of patients in a multidisciplinary environment with appropriate surgical technique and effective haemostatic control. Moreover, the clinical response to BPA may be variable between patients. The optimal use of BPA is hampered by a lack of laboratory assays to monitor efficacy and determine adequate dosing. Recently, we described the first application of TGT in a surgical setting showing that the assay might be useful in guiding the choice of the most effective therapeutic option in inhibitor patients(5). We report now a prospective clinical assessment of TGT and our results show a correlation between thrombin generation (TG) capacity and the clinical outcome of patients.

MATERIALS and METHODS

Subjects & Surgical Procedures: The correlation between the clinical and the biological efficacy of BPA measured by TGT was prospectively evaluated in ten surgical procedures. Six patients with severe hemophilia A and high titer inhibitors (>5 BU/mL), undergoing elective surgeries were treated and documented after obtaining informed consent in accordance with the Declaration of Helsinki. The study was approved by the Lyon University Hospital's ethical committee.

The control population comprised 96 healthy males (40.2 years±11; mean±SD), not using drugs known to affect the coagulation system and without history of bleeding or thrombosis.

Study Design: Dose tailoring of BPA was performed using a standardised three-step-protocol including i) in-vitro spiking experiments evaluating the TG ability of increasing concentrations of NovoSeven® (0-90-180-240-270 µg/kg) and Feiba® (0-75-100 U/kg) in order to determine the minimal dose of each BPA that normalizes TG capacity; ii) ex-vivo confirmation step where TG is measured before and after the administration of the BPA which gave the best haemostatic profile in the previous in-vitro spiking experiment using the dose which fully normalized in-vitro TG and iii) monitoring of the chosen dose of the BPA during the surgery and postoperative period.

As NovoSeven® induces FXa and FIXa generation on activated platelets (6, 7), the haemostatic efficacy of NovoSeven® was evaluated using platelet rich plasma (PRP) (8). Feiba, having a different mechanism of action, was evaluated using platelet poor plasma (PPP) (9).

Blood and PPP&PRP Samples: Venous blood was collected into citrated Monovette® tubes (Sarstedt, Orsay, France) loaded with corn trypsin inhibitor 1.45µM (Haematologic Technologies, USA). PPP and PRP were obtained as previously described (10-12).
Routine measurements: FVIII activity was measured using BioMerieux-deficient-FVIII kit (Marcy l’Etoile, France). Anti-FVIII antibody levels were determined by the Bethesda assay as previously described (13).

TG measurement: TG was measured using the calibrated automated TGT (CAT, Thrombinoscope bv, Maastricht, The Netherlands) and a Fluoroscan Ascent fluorometer (Thermolabsystems OY, Helsinki, Finland) as previously described (10-12), using TF1pM and phospholipids 4µM (final concentrations). PRP samples were tested with TF1pM only. Results were available in three hours following blood drawing. The analysis of the main TGT parameters i.e. peak, ETP (endogenous thrombin potential) and TG-rate, showed a certain correlation between clinical bleeding phenotype and both ETP and TG-rate. However, the large variability of TG-rate in normal control PRPs makes it difficult to reliably determine normal values for this parameter. The peak can not be chosen as the peak values obtained in PRP cannot be compared to those obtained in PPP. In this study, we therefore used ETP as the main biological parameter.

RESULTS and DISCUSSION
Normal ETP values determined in 96 controls were 1487±186nM.min (mean±SD) in PPP and 1544±178nM.min (mean±SD) in PRP.

Table 1 summarizes patient’s characteristics, surgeries, ETP obtained with the three-step-protocol, BPA regimen and clinical outcome for each patient. TG was systematically measured before and one hour after each infusion of BPA during the surgery and every morning before a new infusion, to evaluate the residual TG activity of the patient. Intraoperative blood loss was quantified by measuring irrigation fluid and weighing surgical sponges used for blood and fluid collection during surgery.

Case #1: The patient was clinically a bad responder to Feiba® and he reported a weaker efficacy of NovoSeven®90µg.kg⁻¹ during his last bleeding episode. According to TGT results, the surgery was performed using NovoSeven®200µg.kg⁻¹. Another high dose of 200µg.kg⁻¹ was infused 2h later, followed by the usual regimen of 90µg.kg⁻¹ every 2h. On the 2nd postoperative day NovoSeven®90µg.kg⁻¹ was given every 3h before starting continuous infusion with a dosage of 20µg.kg⁻¹.h⁻¹. The patient exhibited a bleeding at postoperative day 3, after the modification of NovoSeven® regimen. At this time, we observed a significant decrease in both ETP and hemoglobin suggesting a correlation between TG capacity and clinical bleeding risk.

Case #2: In-vitro spiking experiments showed a similar satisfactory efficacy of both NovoSeven® and Feiba®. The patient’s usual home treatment was Feiba® for which the ex-vivo assessment confirmed haemostastic efficacy. Surgery was performed under Feiba®75U.kg⁻¹ given every 8h with no excessive bleeding.

Case #3: The patient underwent four orthopedic procedures during the study period. The standardized three-step protocol was performed before each surgery. Results were convergent and they showed a
better efficacy of Feiba® in this particular patient. Ex-vivo TG measurements performed before each surgery confirmed a complete correction of ETP with Feiba® 75U·kg⁻¹. The patient underwent the four surgeries with Feiba® 75U·kg⁻¹ every 8h with satisfactory clinical efficacy.

**Case #4:** The patient was a poor responder to both Feiba® and NovoSeven® with unsatisfactory bleeding control with these drugs. In-vitro and ex-vivo TG measurements were performed before the surgery and confirmed the insufficient correction of TG in this particular patient with both Feiba® or NovoSeven®. With the knowledge of these results and considering the extremely high risk of the surgery, we designed a strategy that included immunoabsorption in order to eliminate the inhibitor, substitution with FVIII and administration of FEIBA® when the anamnestic response occurred. The monitoring of this successful strategy using TGT throughout the perioperative period was previously described in a case report(5).

**Case #5:** The patient underwent two surgical procedures during the study period. Before the cataract surgery in-vitro spiking experiments showed insufficient TG correction with Feiba® and NovoSeven® at all tested concentrations. However, a partial correction was observed in the presence of NovoSeven® 120µg·kg⁻¹ (ETP = 815nM·min⁻¹), with a plateau reached beyond 120µg·kg⁻¹. He underwent the cataract surgery with NovoSeven® 120µg·kg⁻¹. No bleeding occurred during the surgery which is usually considered safe regarding the bleeding risk. Eight months later, he was admitted to the emergency department for acute rectal bleeding. He was first treated with NovoSeven® 90µg·kg⁻¹ every 2h; he experienced continuous bleeding despite NovoSeven® infusions. Colonoscopy identified a dissecting intramural haematoma of the sigmoid colon. The patient underwent urgent bowel resection. The surgery was performed after an infusion of NovoSeven® 120µg·kg⁻¹ which was repeated every 2h for 48 hours. The patient exhibited life threatening bleeding despite bypassing therapy and he was intensively transfused. On the 5th postoperative day, rebleeding occurred. CT scan demonstrated concomitant intra-peritoneal and intra-pleural bleeding. The patient’s condition rapidly deteriorated and NovoSeven® was stopped and replaced by Feiba® 100U·kg⁻¹. In parallel, the patient was intensively transfused but clinical efficacy could not be obtained. The patient developed disseminated intravascular coagulation (DIC) and death occurred the day after as a result of continuous hemorrhage. In this particular patient neither NovoSeven® nor Feiba® could achieve effective hemostasis as demonstrated by deficient TG. The concomitant use of both drugs was not tested in this patient because of DIC(14).

**Case #6:** Before synovectomy in-vitro spiking experiments showed a better efficacy of NovoSeven® 90µg·kg⁻¹ compared to Feiba® 75U·kg⁻¹. Ex-vivo results confirmed the haemostatic efficacy of NovoSeven® 90µg·kg⁻¹ which normalized the ETP values one hour after infusion. The synovectomy was performed after an infusion of NovoSeven® 90µg·kg⁻¹. No bleeding complication occurred.

The treatment and monitoring of BPA is still challenging and there is a need for a reliable biomarker which correlates with the clinical outcome of patients. This study was designed to prospectively assess a three-step-protocol using TGT which aims to individually tailor and monitor BPA in surgical
situations. We observed that in patients with normalized ETP, no bleeding complication occurred. We conclude that TGT results correlate with the surgery related clinical bleeding risk and ETP may be used as a surrogate marker for monitoring BPA. Careful standardization of the preanalytical and analytical test conditions is required before a wider application of the assay in clinical laboratories (15).

REFERENCES


Acknowledgements and Disclosures

This work was supported by the Bayer Hemophilia Awards Program.

CN has participated in advisory committee meetings, and received research funding, consultancy and honoraria from Baxter and Novo Nordisk. YD received research funding and speakers honoraria from Novo Nordisk and Baxter.

Authorship

Yesim Dargaud designed, performed the research project and wrote the manuscript
Anne Lienhart performed the study
Claude Negrier designed the project and revised the manuscript
**Table 1A**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Surgical procedure</th>
<th>Treatment regimen</th>
<th>ETP during surgery (nM/min)</th>
<th>Intra-operative blood loss (mL)</th>
<th>Hemoglobin (g/dL)</th>
<th>Clinical outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Transmetatarsal amputation of the left low limb</td>
<td>D0: rFVIIa 200 µg/kg</td>
<td>1567 (normalized)</td>
<td>300</td>
<td>12.1</td>
<td>12.1</td>
</tr>
<tr>
<td>2</td>
<td>Left total ankle arthroplasty</td>
<td>A P C C 75 U/kg/8H</td>
<td>1440 (normalized)</td>
<td>500</td>
<td>15/12</td>
<td>No excessive bleeding and satisfactory wound healing</td>
</tr>
<tr>
<td>3</td>
<td>Right elbow synovectomy</td>
<td>A P C C 75 U/kg/8H</td>
<td>1417 ± 164 (mean±SD, n=6)</td>
<td>170</td>
<td>12/12</td>
<td>No excessive bleeding and satisfactory wound healing</td>
</tr>
<tr>
<td>4</td>
<td>Bilateral total knee arthroplasty</td>
<td>Immunoaffinity removal FVIII concentrate</td>
<td>A P C C 75 U/kg at D4</td>
<td>600 x2</td>
<td>12/7</td>
<td>No excessive bleeding and satisfactory wound healing</td>
</tr>
<tr>
<td>5</td>
<td>Cataract surgery</td>
<td>rFVIIa 120 µg/kg</td>
<td>595 (low ETP)</td>
<td>0</td>
<td>13/13.1</td>
<td>No excessive bleeding and satisfactory wound healing</td>
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<td>6</td>
<td>Left elbow synovectomy</td>
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**Step 1: In vitro spiking experiments – ETP (nM.min)**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>FVII:C (%)</th>
<th>Inhibitor (BU/ml)</th>
<th>Clinical BPA response of patients before TG testing</th>
<th>rFVIIa 0 µg/kg</th>
<th>rFVIIa 50 µg/kg</th>
<th>rFVIIa 100 µg/kg</th>
<th>rFVIIa 200 µg/kg</th>
<th>A P C C 100 U/kg</th>
<th>A P C C 75 U/kg</th>
<th>Chosen ETP</th>
<th>Chosen dose</th>
<th>ETP 1h after infusion (nM.min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>&lt;1</td>
<td>200</td>
<td>Good responder to A P C C and r F VI I a</td>
<td>315</td>
<td>1067</td>
<td>1375 (normalized)</td>
<td>1395 (normalized)</td>
<td>288</td>
<td>663</td>
<td>1012</td>
<td>rFVIIa</td>
<td>200 µg/kg</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>&lt;1</td>
<td>18</td>
<td>Good responder to A P C C</td>
<td>410</td>
<td>1650</td>
<td>2337</td>
<td>2304</td>
<td>1356</td>
<td>1376</td>
<td>2076</td>
<td>A P C C</td>
<td>75 U/kg</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>&lt;1</td>
<td>9</td>
<td>Good responder to A P C C</td>
<td>164</td>
<td>505</td>
<td>1004</td>
<td>1051</td>
<td>110</td>
<td>1256</td>
<td>1350</td>
<td>rFVIIa</td>
<td>120 µg/kg</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>&lt;1</td>
<td>78</td>
<td>Good responder to both A P C C and r F VI I a</td>
<td>180</td>
<td>283</td>
<td>344</td>
<td>447</td>
<td>187</td>
<td>634</td>
<td>896</td>
<td>A P C C</td>
<td>75 U/kg</td>
</tr>
<tr>
<td>5</td>
<td>71</td>
<td>&lt;1</td>
<td>10</td>
<td>Good responder to A P C C and r F VI I a</td>
<td>180</td>
<td>462</td>
<td>626</td>
<td>644</td>
<td>186</td>
<td>868</td>
<td>930</td>
<td>rFVIIa</td>
<td>120 µg/kg</td>
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<tr>
<td>6</td>
<td>33</td>
<td>&lt;1</td>
<td>24</td>
<td>Good responder to A P C C</td>
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<td>1550</td>
<td>1690</td>
<td>1943</td>
<td>199</td>
<td>1004</td>
<td>1054</td>
<td>rFVIIa</td>
<td>90 µg/kg</td>
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**Step 2: Ex vivo confirmation**

**Step 3: Monitoring during surgery**

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**Table 1B**
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