

BRIEF REPORT

Alternative treatment options for pediatric hemophilia B patients with high-responding inhibitors: A thrombin generation-guided study

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Abstract

Little is known about the challenging treatment of pediatric patients with hemophilia B and inhibitors due to disease rarity. We describe three patients diagnosed in childhood and followed up to 9 years. All three had allergic reactions to Factor IX, but two were later safely treated for bleeding episodes with activated prothrombin complex concentrates (APCC = FEIBA). The third was given only recombinant activated Factor VIIa. Based on ex vivo thrombin generation analysis, a new alternative treatment of combined bypassing agents was administered for bleeding episodes and several minor surgical procedures with no treatment-associated adverse events or thrombosis.

KEYWORDS

FEIBA, hemophilia B, inhibitor, rFVIIa, thrombin generation

1 | INTRODUCTION

Hemophilia B (HB) is a congenital severe bleeding disorder characterized by Factor IX (FIX) deficiency. Recent gene therapy studies have described the development of potential cures for HB¹; however, the current standard of care continues to be replacement therapy.^{1,2} Unfortunately, the formation of antibodies is a complication that renders such care ineffective. Inhibitors affect 30% of patients with severe hemophilia A (HA), but they are much more rare (1.5%–3%) in patients with HB.³ FIX inhibitors are associated with significant morbidity due to life-threatening allergic reactions that may precede the development of inhibitors in 50% of patients, usually in childhood.⁴ Furthermore, immune tolerance induction (ITI) is less effective in HB compared with HA.⁵ Unlike HA patients who necessitate bypassing agents (BPA) consisting of recombinant activated Factor VII (rFVIIa) or activated prothrombin complex concentrate (APCC, e.g., Factor VIII inhibitor bypassing activity [FEIBA]) therapy solely for high-titer inhibitors.⁶ HB patients may require BPA regardless of inhibitor titer due to previous anaphylaxis⁷ and nephrotic syndrome⁸ associated with exposure to FIX. The choice of a suitable therapy may be complicated, because there are only a few small-scale reports that provide

potential guidance for the care of FIX inhibitor patients. Given the difference between HA and HB with regard to inhibitor epidemiology and natural history, the guidelines for optimal care of HA inhibitor patients do not necessarily apply to FIX inhibitor patients.³

The motivation for conducting this study was an adolescent HB patient with long-standing high-titer inhibitor (peak measured at 28 BU) who requested circumcision surgery. This patient was diagnosed at 6 years of age at another center and treated in our clinic since the age of 10 years. He reportedly had FIX allergy (allergic rash, no anaphylaxis), although no desensitization or ITI had been attempted. He had been considered a “poor responder” to both rFVIIa and FEIBA at maximal therapeutic doses for his recurrent hemarthroses. Thrombin generation (TG) studies⁹ were performed with ex vivo BPA spiking in an attempt to tailor his optimal perisurgical therapy, and the results revealed that combined rFVIIa–FEIBA could serve as an optional treatment. This led us to conduct a more comprehensive ex vivo TG study on three HB patients with inhibitors who were treated with combined rFVIIa–FEIBA and followed up in our National Hemophilia Center and Thrombosis Unit.

2 | RESULTS

Three HB patients (FIX < 1%) with inhibitors were studied. Their demographic data and characteristics are presented in Table 1, and further

Abbreviations: APCC, activated prothrombin complex concentrate; BPA, bypassing agents; FEIBA, factor eight inhibitor bypassing activity; FIX, factor IX; HA, hemophilia A; HB, hemophilia B; ITI, immune tolerance induction; rFVIIa, recombinant activated Factor VII; TG, thrombin generation

TABLE 1 Demographic and clinical characteristics of patients

Patient no.	Age (years)	F9 gene mutation	Inhibitor (BU)	ABR	Target joint
1	18	Deletion (frameshift): c. 848_849delAT p.Asn283Asn fs stop+1	0.7–28	25	Ankle, elbow, knees
2	13.5	Nonsense: c.115A > T p.Lys39 stop	0.5–24	23	Elbow, knees
3	2.5	Nonsense: c.54C > A p.Cys18 stop	3–74	20	no

ABR, annual bleeding rate; median values are provided.

details together with reagents and laboratory studies are provided in Supporting Material S1. They were all at high risk for FIX inhibitor formation due to their gross or complete gene deletions and severe bleeding phenotype (Table 1).

The need to perform semi-elective surgery in our “difficult-to-treat” high-responding inhibitor impetus adolescent (patient 1) led us to evaluate the potential role of standard therapy as well as combination therapies on patients’ plasma samples tested by TG. Spiking with FIX (100% = U mL⁻¹), rFVIIa (2.25 and 6.75 µg mL⁻¹ [≈80 and ≈240 µg kg⁻¹]), and FEIBA (0.6 and 1.2 U mL⁻¹ [≈50 and 100 IU kg⁻¹]) alone or in combination was performed simultaneously on all three patients’ samples. Figure 1A–C shows recalcification-induced TG curves of patients 1 to 3, respectively. Flat baseline TG curves were detected in all samples without additional exogenous BPA. Increased rFVIIa or FEIBA concentrations directly correlated with increased TG in all samples. TG induced by the combination of low concentrations of rFVIIa plus FEIBA (2.25 µg mL⁻¹ and 0.6 U mL⁻¹, respectively) led to similar responses as a higher concentration of either rFVIIa (6.75 µg mL⁻¹) or FEIBA (1.2 U mL⁻¹) alone.

The maximal ETP detected for patients 1, 2, and 3 were 524.1, 555.3, and 438.7 nM min, and the maximal peak heights were 32.2, 31.7, and 21.5 nM, respectively. However, the patients’ ETP and peak height levels were significantly lower compared with normal controls, i.e., 1915 ± 641 nM min and 342.6 ± 172 nM, respectively. Following our experience with combined bypass agents therapy for HA patients with inhibitors¹⁰ and based on the TG analysis findings, the circumcision was performed with the patient receiving combined FEIBA: 40 IU kg⁻¹ + rFVIIa 80 µg kg⁻¹ at decreasing frequencies. Specifically, it was administered every 6 hours on the day of surgery, reduced to thrice and twice daily, and discontinued on postoperative day 5 with no bleeding complications. Analysis of the patient’s plasma before and 20 minutes after combined BPA showed a dramatic elevation in TG (Figure 1D). Because the hemostatic response was satisfactory and no complication had been detected during the perioperative period, we started treating patient 1 for bleeding episodes with FEIBA 30 to 50 IU kg⁻¹ + rFVIIa 50 to 100 µg kg⁻¹. He required 1 to 3 injections every 6 to 12 hours per bleeding event (most events occurred in target joints) until resolution. Patient 2 was initially treated with rFVIIa at 90 µg kg⁻¹. Due to

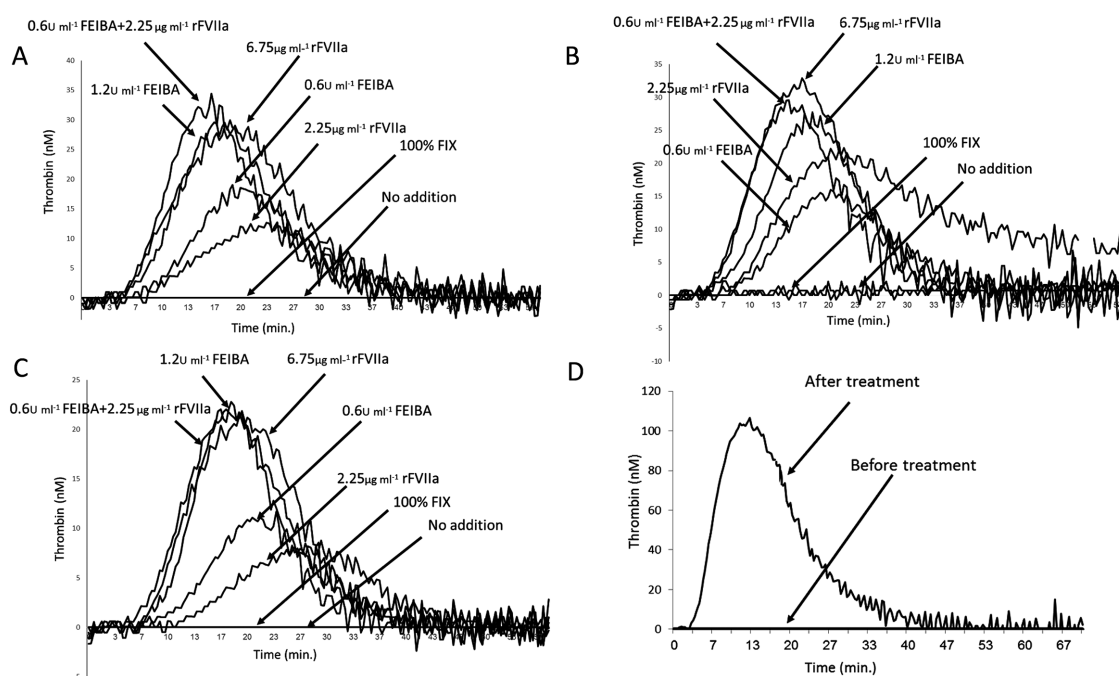


FIGURE 1 TG analysis as a tool for assessing the potential use of combined FEIBA and rFVIIa for hemophilia B patients with inhibitor. A–C, Representative TG curves of patients 1 to 3, respectively. The patients’ PPP was spiked ex vivo with FIX, rFVIIa, FEIBA alone or in combination. D, TG was analyzed in plasma taken from patient 1 before and 20 minutes after in vivo treatment with FEIBA (40 IU kg⁻¹) + rFVIIa (80 µg kg⁻¹). The flat curve obtained prior to treatment shows significant improvement once combined concomitant rFVIIa + FEIBA were administered

a poor response to increasing doses up to $270 \mu\text{g kg}^{-1}$, therapy was switched to FEIBA prophylaxis (80 IU kg^{-1} , thrice weekly). There was no allergic reaction; however, breakthrough bleeding episodes did not respond well to additional FEIBA dosing. The patient's parents refused ITI. Given our TG results as well as our clinical experience with patient 1, we started treating these episodes with a lower FEIBA dose ($30\text{--}50 \text{ IU kg}^{-1}$) along with rFVIIa 50 to $100 \mu\text{g kg}^{-1}$. His annual bleeding rate has recently decreased from 23 to 8 episodes. Patient 2 also underwent several minor surgeries that included meatotomy, radioactive synovectomy of knee, and three port-a-cath insertions (2 extractions due to mechanical obstruction, tear or infection) under FEIBA 45 IU kg^{-1} + rFVIIa $90 \mu\text{g kg}^{-1}$ thrice daily followed by twice daily for another 2 days, without complications. A comparison of TG plots of patient 2 before and after combined BPA showed an increased TG, supporting the beneficial hemostatic effect of the combined treatment. Patient 3, who developed an inhibitor at infancy after only 5 exposure days following anaphylactic reaction to recombinant FIX, had no desensitization due to his young age and is currently being treated with rFVIIa $270 \mu\text{g kg}^{-1}/\text{dose}$, to which he responds well. The combined therapy protocol could not be applied in this case because of his past FIX anaphylaxis.

All three patients have been followed for up to 9 years to date. There have been no severe adverse events or any thrombosis despite extensive bypass therapies.

3 | DISCUSSION

According to the HB database, 23% of patients with gross gene deletions and 30% with complete gene deletions are at risk to develop inhibitors.^{3,12} We describe three pediatric patients out of 100 patients with HB followed in our center, a figure compatible with the inhibitor incidence in the literature. They were all at high risk for inhibitor formation due to their high-risk mutations. Knowledge about the optimal treatment of these patients is lacking because data on inhibitors in pediatric HB patients are very scarce.³ Only few studies have addressed treatment or prevention of bleeding episodes involving patients with HB and inhibitors. As a result, most standards of care using FIX ITI with immune modulation, rFVIIa, or FEIBA, are derived from the studies on patients with FVIII inhibitor. In the current study, based on the results of an ex vivo TG analysis, a new alternative treatment of combined BPA administration was applied to two of our three patients for bleeding episodes and minor surgeries. Notably, repeated administration of combined BPA was safe, and there were no severe adverse events or any documented thrombosis among our patients. Despite the potential use of combined low-dose BPA to restore hemostasis in all patients, patient 3 (who presented with anaphylactic reaction to recombinant FIX) is being treated with rFVIIa only.

The current report describes our experiences with the use of TG as an ancillary therapeutic tool for aiding therapy tailoring and monitoring. Combined BPA therapy, which had previously been applied only for HA patients with inhibitors^{11,13} may become an additional option

for the treatment of some HB patients with inhibitors. The current choice of treatment considers the severity of bleeding episodes, the FIX inhibitor titer, and any allergic responses to FIX. In the era where the complexity of these patients' treatment may be resolved by novel nonreplacement drugs,¹⁴ there is a special unmet need to individually tailor treatment. Our data may be of future use for hemophilia treaters facing similarly challenging situations.

CONFLICTS OF INTEREST

G.K. received research grants from Baxalta, Bayer, Pfizer, Opko Biologics, took part in hematology advisory boards of Novonordisk and Bayer companies and served as PI in clinical studies sponsored by these companies and as consultant for Bayer, Opko Biologics, Pfizer, Alnylam. S.L.-M. served as consultant for Baxalta and PI for Bayer studies. M.M. served as PI for Novonordisk studies. The other authors have no competing interest to disclose.

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AUTHOR CONTRIBUTIONS

The authors have made the following declaration about their contribution:

Livnat T, Barg AA and Kenet G: Conceived and designed the experiments.

Livnat T, Avishai E and Kenet G: Performed experiments.

Livnat T, Barg AA, Levy-Mendelovich S and Kenet G: Analyzed the data.

Misgav M, Livnat T, Levy-Mendelovich S, Barg AA and Kenet G: Provided resources for experiments.

Kenet G, Barg AA, Levy-Mendelovich S and Livnat T: Wrote the paper

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Supplement Material S1.

Materials and methods

Patients and ethics

Three HB patients (FIX <1%) with inhibitors, part of the patient population followed in our center, were studied. Demographic data and patients' characteristics are presented in Table 1. Laboratory studies were performed during routine clinic visits. Control plasma samples for laboratory assays were obtained from healthy male volunteers. Patients who were treated by BPA during clinic visits or hospitalization were daily monitored and post treatment TG samples were obtained and studied as compared to pre-treatment samples. Home treated bleeding episodes were reported to the investigator and response was assessed by a telephone call conducted by the physician within 24 hours of therapy application.

All subjects or their guardians gave their informed consent to blood drawing for study purposes. The study was approved by the institutional ethical committee in compliance with the Declaration of Helsinki.

Reagents

rFVIIa was purchased from NovoNordisk, Bagsvaerd, Denmark. FIX (Immunine) and FEIBA were purchased from Baxter, Vienna, Austria. The reagents were prepared according to the manufacturer's instructions.

Processing of blood samples

Blood samples were drawn in 0.109 M buffered citrate tubes. Platelet-poor plasma (PPP) was obtained at room temperature by centrifugation of blood at 2000 g for 10 min. The plasma was then aspirated into a plastic tube, and residual platelets were removed following a further centrifugation at 14000 g for 5 min. The double-spin PPP was stored in aliquots at -70°C and thawed prior to assay.

Thrombin generation

TG was measured in PPP as described previously [9]. Twenty microliters of working buffer containing 4 μ M phospholipid with or without 1pM TF (PPP-Reagent low or MP-Reagent respectively, Stago, Gennevilliers, France) were placed in round-bottom 96-well plates. Eighty microliters of plasma were added to the buffer. FEIBA, rFVIIa and FXI alone or in combination were spiked into the plasma [10]. TG was initiated by adding 20 μ L of fluorogenic substrate/ CaCl_2 buffer (FluCa-kit, Stago).

Fluorescence was measured with a fluorometer (Fluoroskan Ascent, Lab system, Helsinki, Finland) with an excitation filter at 390 nm and an emission filter at 460 nm. The results were displayed as plots and derived parameters, i.e., lag time (min), endogenous thrombin potential (ETP, nM.min) and peak height (nM), calculated by dedicated computer software attached to the fluorometer (version 3.0.0.29 Thrombinoscope-BV). All experiments were repeated by the same researcher.