

Thrombogenicity evaluation in 221 patients with haemophilia B treated with nonacog alfa

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Risk for thrombotic events with factor IX replacement therapy in patients with haemophilia B remains a concern for patients, those who treat them, and regulatory agencies, based on experience with early use of prothrombin complex concentrates. The current post hoc analysis assessed the incidence of thrombotic events and changes in prothrombin fragment 1 + 2, thrombin–antithrombin complex, and D-dimer in 221 patients with haemophilia B who received nonacog alfa in clinical studies. Thrombotic event and coagulation marker data were collected from 8 interventional studies utilizing on-demand, prophylactic, and preventive regimens in patients with haemophilia B. Mean age was 25 years (min–max, 0–69), with 51 (23%) patients aged less than 12 years and 15 (7%) aged less than 2 years. None tested positive for inhibitors. Mean time on study was 60.9 ± 32 weeks and mean number of exposure days was 69.3 (min–max, 1–496). Sixty-nine (31%) patients regularly received infusions that were approximately 100 IU/kg as part of a routine prophylaxis regimen, and 29 (13%) patients underwent surgical procedures. No clinical thrombotic events were reported, and no patient experienced clinically significant changes in coagulation

markers between baseline and end-of-study testing. These collective data support the low thrombotic risk associated with nonacog alfa in paediatric, adult, and surgical patients with haemophilia B receiving different treatment regimens, including doses of approximately 100 IU/kg. Although careful thrombotic clinical evaluation is important, regular coagulation marker monitoring does not appear to be warranted in patients with haemophilia B. *Blood Coagul Fibrinolysis* 29:81–86 Copyright © 2018 The Author(s). Published by Wolters Kluwer Health, Inc.

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Introduction

Haemophilia B is an inherited, X-linked bleeding disorder caused by a deficiency of coagulation factor IX (FIX) activity. In affected individuals, repeated spontaneous or trauma-induced bleeding into joints and soft tissue can lead to painful, chronic joint disease and potentially life-threatening intracranial or gastrointestinal bleeding events [1]. The standard of care involves the replacement of missing or defective FIX with plasma-derived or recombinant FIX products and, more recently, with recombinant, extended half-life FIX products, which may be administered for the on-demand treatment of acute bleeding episodes or as prophylactic treatment to reduce the risk of haemorrhagic events [1,2].

Almost 50 years ago, plasma-derived prothrombin complex concentrates (PCCs) were introduced for the management of haemophilia B, leading to dramatic improvements in the quality of life [1,3] of patients with the disease. However, a report in 1977 gave an incidence of thrombotic events in 10/109 (9.2%) patients with haemophilia B [4]. The PCC replacement corrected the FIX deficiency but resulted in supraphysiologic concentrations of the other factors because PCCs also contained factors VII and X [5]. The thrombogenicity of PCC

treatment was attributed to the accumulation or delayed clearance of unactivated and activated vitamin K-dependent proteins [6,7], and the risk of thrombogenicity appeared to be increased by the presence of large muscular haematomas, orthopaedic surgery, or the presence of inhibitors, which required large, frequent doses of PCCs [7,8]. Subsequently, PCCs were replaced by high-purity, plasma-derived FIX concentrates and human recombinant FIX, which, by virtue of specific component makeup, significantly reduce and/or eliminate the risk of viral transmission and do not trigger activation of the coagulation cascade, thereby reducing thrombotic risk [1,9].

Several biomarkers are associated with the coagulation process. Prothrombin fragment 1 + 2 (F_{1+2}) is generated by factor Xa in the cleavage of prothrombin to thrombin, and thrombin–antithrombin (TAT) complex is formed by the combination of thrombin and its primary inhibitor, antithrombin. Although F_{1+2} and TAT reflect the degree of thrombin activation, D-dimer, a high-molecular-weight fibrinogen compound derived from the cleavage of cross-linked fibrin, reflects fibrinolytic activity and, when elevated, may indicate the presence of intravascular thrombus [10]. There is significant interpersonal

variability in D-dimer levels within the normal range in healthy individuals [11]. Elevated levels occur in various disorders in which the coagulation system is excessively activated [12–14].

The risk of thromboembolism with FIX replacement therapy remains a concern for haemophilia patients, their treating physicians, and relevant regulatory agencies. In July 2011, the European Medicines Agency began to require the monitoring and reporting of thrombotic events in clinical studies of the new recombinant and human plasma-derived FIX products. In addition, the appropriate testing for biomarkers of activated coagulation and fibrinolysis (F_{1+2} , TAT, and D-dimer) before and after FIX infusions is required [15]. The United States Food and Drug Administration also recommends the evaluation of coagulation markers in clinical trials of all FIX products.

Nonacog alfa was the first recombinant FIX product licensed in the United States in 1997 and in Europe in 1998 for the control and prevention of haemorrhagic episodes and for routine and surgical prophylaxis in adult and paediatric patients with haemophilia B [16,17]. Many clinical studies spanning 18 years established the efficacy and safety of nonacog alfa across a range of patient populations, including previously treated and untreated children and adults, in on-demand, prophylactic, and surgical settings, and with the use of various doses [18–27]. The reporting of thromboembolic events was required in all of the studies, and the prospective monitoring of circulating markers of activated coagulation and fibrinolysis was required in some of the pivotal studies. The purpose of this analysis was to assess the incidence of thrombotic events, changes in blood coagulation markers, and the utility of collecting these data in patients with haemophilia B who were receiving nonacog alfa (BeneFIX; Pfizer Inc, Philadelphia, Pennsylvania, USA) and participating in these clinical trials.

Methods

The current post hoc, retrospective, pooled analysis included patients with haemophilia B who received at least one infusion of nonacog alfa in eight interventional clinical studies, all sponsored by Wyeth/Pfizer Inc [19–21,24–27] (Table 1). The studies were registered at clinicaltrials.gov with identifiers NCT00037557 (Study 301), NCT00093171 (Study 302), NCT00093210 (Study 304), NCT00364182 (Study 400), and NCT00167973 (Study 1010). As Studies 200, 201, and 202 were conducted before trials were required to be registered, no identifiers are available for those studies. All of the studies were conducted in accordance with the principles of the Declaration of Helsinki and with local regulations, and each study protocol was approved by an ethics committee.

Selection of the interventional studies was based on the consideration of a high risk of thrombotic events (i.e., patients who received nonacog alfa infusions of at least 100 IU/kg and patients who underwent surgical procedures) and on the different characteristics of the patient population [i.e., age, number of exposure days, and treatment modality (on-demand, preventive, or prophylactic)]. The study designs and the efficacy and safety results for all studies were previously reported [19–21,24–27]. The doses of nonacog alfa were determined by investigators in accordance with protocol guidance and/or the approved labelling, with the exception of Study 400, wherein patients received two prophylaxis regimens (nonacog alfa 50 IU/kg twice weekly and 100 IU/kg once weekly) [24], and Study 1010, wherein patients were to receive a prophylaxis regimen of nonacog alfa 100 IU/kg once weekly [25]. Thrombotic events were defined as any event associated with the formation of a blood clot, including catheter-associated thrombi and thrombotic complications in treated patients, and were considered protocol-specified, medically important events subject to reporting. In this pooled analysis, the total number of clinically observed thromboembolic events in all studies was collected.

Sample collection for coagulation marker assessment, including partial thromboplastin time, F_{1+2} , TAT, and D-dimer, was conducted while in a nonbleeding state; sampling for F_{1+2} required a separate venepuncture. The samples included in this analysis were collected before and after infusion on day 1 and at the final study visit. For patients undergoing surgical procedures, blood samples were drawn for the assessment of coagulation markers immediately prior to and after the loading dose, and during the immediate postoperative period (inpatient postoperative period) for as long as the patient was receiving nonacog alfa. Blood coagulation markers were assessed by a central laboratory in each study.

Data were analysed using descriptive statistics, including means, medians, ranges, and SDs.

Results

A total of 221 patients received at least one dose of nonacog alfa in the eight interventional studies and were included in this analysis. The mean age (SD) at screening was 25 (± 16) years, with 51 (23%) of the patients younger than 12 years of age and 15 (7%) younger than 2 years of age. The majority of patients were male (99%) and white (85%) (Table 2). The two female patients were carriers and were enrolled in Study 200. No patient tested positive for inhibitors to FIX. Patients were on study for a mean of 60.9 (± 32) weeks, with a mean of 69.3 exposure days (Table 2). The analysis included 69 (31%) patients who regularly received infusions that were approximately 100 IU/kg as part of a routine prophylaxis regimen and 29 (13%) patients who underwent surgical procedures.

Table 1 Summary of the nonacog alfa studies included in this pooled analysis

Study	Design	Patients, n	Objective	Patient population	Inclusion criteria	Thrombogenicity evaluation
200 [26]	Open-label, nonrandomized study	45	Evaluate efficacy and safety	PTPs with moderate or severe haemophilia B	FIX:C \leq 5%	Prothrombin fragment 1 + 2: 1 h before infusion, between 4–8 and 24 h after infusion
201 [26]	Double-blind, randomized, crossover PK study	11	Evaluate efficacy and safety	PTPs with moderate or severe haemophilia B	FIX:C \leq 5%	Prothrombin fragment 1 + 2: 1 h before infusion, between 4–8 and 24 h after infusion
202 [19]	Open-label, nonrandomized, multicenter study	9 ^a	Evaluate efficacy and safety in elective surgical setting	PTPs aged \geq 3 years with mild, moderate, or severe haemophilia B	FIX:C \leq 1% to $>$ 10%; scheduled to undergo elective surgery	Partial thromboplastin time: before and 30 min after infusion Prothrombin fragment 1 + 2: before infusion, between 4 and 8 h after infusion, and 24 h after infusion
301 [21]	Open-label, nonrandomized, multicenter study	25	Evaluate efficacy and safety in treatment of acute bleeding episodes, prophylaxis, and/or surgery	Children aged $<$ 6 years with severe haemophilia B	FIX:C \leq 1%; aged $<$ 5 years at baseline to allow for study treatment completion before age 6	TAT, D-dimer, prothrombin fragment 1 + 2: screening, before and after infusion on day 1, at months 1 and 3, every 3 months thereafter while patient was on study, and at final visit For surgical procedures: 15 \pm 3 days after procedures TAT, D-dimer, prothrombin fragment 1 + 2: before infusion and at final visit
302 [27]	Open-label, nonrandomized, multinational study	23	Evaluate efficacy and safety during standard of care treatment (on-demand, prophylaxis, and surgical setting)	PTPs aged \geq 12 years with moderately severe or severe haemophilia B	FIX:C \leq 2%; \geq 150 exposure days	For surgical procedures: before and after infusion at 30 min and at 4, 8, and 24 h, and a minimum of once daily thereafter Activated partial thromboplastin time, TAT, D-dimer, prothrombin fragment 1 + 2: Baseline, over a 72-h pharmacokinetic sampling period, and at 6, 9, and 12 months
304 [20]	Double-blind, randomized, crossover PK study followed by a 6–12-month open-label, on-demand treatment extension	34	Determine bioequivalence of new formulation to reference formulation; evaluate efficacy and safety of new formulation	PTPs aged \geq 12 years with moderately severe or severe haemophilia B	FIX:C \leq 2%; \geq 150 exposure days	No routine assessment of coagulation markers; assessment based on clinical symptoms
400 [24]	Open-label, randomized, 4-period, crossover study	49	Evaluate efficacy and safety of 2 prophylaxis regimens vs. on-demand treatment	PTPs aged 6–65 years with moderately severe or severe haemophilia B	FIX:C \leq 2%; \geq 12 bleeding episodes (6 of which were joint bleeding events) in the 12 months before screening	
1010 [25]	Prospective, open-label, multicenter study of 6-month on-demand treatment period followed by 12-month prophylaxis period	25	Evaluate efficacy and safety of a prophylaxis regimen vs. on-demand treatment only	PTPs aged 12–65 years with moderately severe to severe haemophilia B	FIX:C \leq 2%; \geq 100 exposure days; \geq 12 bleeding episodes (6 of which were joint bleeding events) in the 12 months before screening	D-dimer and TAT: before and after infusion, at day 1, and at 26 weeks

FIX:C, factor IX activity; PK, pharmacokinetic; PTPs, previously treated patients; TAT, thrombin-antithrombin complex. ^a n = 10 for Study 202; one patient who participated in both Studies 200 and 202 was only counted in Study 200.

Table 2 Baseline demographics and characteristics

Characteristic	Pooled population, <i>n</i> = 221
Age category, <i>n</i> (%) (years)	
Infant (<2)	15 (7)
Child (3–12)	36 (16)
Adolescent (13–16)	25 (11)
Adult (17–64)	143 (65)
Geriatric (≥65)	2 (1)
Age (years)	
Mean (SD)	25 (16)
Median (min–max)	22 (0–69)
Sex, <i>n</i> (%)	
Male	219 (99)
Female	2 (0.9)
Race, <i>n</i> (%)	
White	188 (85)
Black	12 (5)
Asian	12 (5)
Other	9 (4)
Weight (kg) ^a	
Mean (SD)	65 (28)
Median (min–max)	70 (8–173)
Height (cm) ^b	
Mean (SD)	159 (30)
Median (min–max)	171 (70–196)
Weeks on study	
Mean (SD)	61 (32)
Median (min–max)	56 (<1–122)
Exposure days to nonacog alfa ^c	
Mean (SD)	69 (64)
Median (min–max)	56 (1–496)

Max, maximum; min, minimum. ^a*n* = 220. ^b*n* = 192. ^cAny commercial nonacog alfa (BeneFIX) infusions that may have been administered during the study are included.

No clinical symptoms of thrombotic events were reported in any of the patients. Coagulation marker assays were to be performed in 6 of the 8 studies, representing 67% of patients, and showed no clinically significant differences between baseline and levels at the final study visit (Table 3). The differences observed in some cases before and after the FIX infusion or during the surgical procedure were not clinically significant and were not associated with thrombotic events (data not shown).

Discussion

The current analysis presented data for 221 patients with haemophilia B who received nonacog alfa while participating in clinical trials. Administration of nonacog alfa was not associated with clinical evidence of thrombosis or, in the subset of patients with available coagulation

marker values, with changes in biomarkers associated with thrombogenicity. The absence of any evidence of thrombogenic events or clinically relevant changes in coagulation markers is especially notable for the 69 (31%) patients who received regular doses of nonacog alfa 100 IU/kg and for surgical patients (*n* = 29; 13%) who may be at higher risk for developing venous thromboembolism in the perioperative and postoperative time periods [28]. The findings of this pooled analysis are consistent with results from individual reports of previous studies in patients with haemophilia B who received high-purity, plasma-derived FIX products, which were not associated with thrombotic events or with clinically significant changes in activation coagulation markers over the course of replacement therapy [29–32].

Thrombotic events have been reported in other studies of nonacog alfa [22,23]. In one of those studies, one patient developed a clot in the intravenous access device, which was judged to be unrelated to nonacog alfa [23]. A second patient, from a registry study, underwent orthopaedic surgery and developed asymptomatic distal deep vein thrombosis after receiving continuous infusion of nonacog alfa for 12 days; this event was considered to be possibly related to treatment [22]. In addition, in a separate study, instances of arterial thrombotic events were reported following FIX infusion [33].

Elevated FIX activity levels more than 150 IU/dl have been associated with increased thrombotic risk in studies that did not include patients with haemophilia B [34]. The mean ± SD peak FIX activity observed in one study in patients with haemophilia B (*n* = 43) who received nonacog alfa 100 IU/kg once weekly was 91.5 ± 28.9 IU/dl (range 1.1–146.0 IU/dl) [24]. However, it is not possible to extrapolate the thrombotic risk from the general population, in whom FIX levels remain relatively constant over time, to patients with haemophilia B receiving nonacog alfa, who have pronounced peak and trough FIX activity.

FVIII activity levels may be increased under stressful situations, such as the perioperative or inflammatory states [35], and it has been noted that patients with high levels of FVIII activity are at increased risk for thrombotic events [36]. In a systematic review conducted by

Table 3 Summary of coagulation markers measured at baseline and at final study visit^a

Parameter	Partial thromboplastin time (s)	Thrombin–antithrombin complex (μg/l)	D-dimer (ng/ml)	Prothrombin fragment 1 + 2 (nmol/l)
<i>n</i>				
Baseline	97	90	92	117
Final	92	98	112	128
Mean (SD)				
Baseline	41 (17)	3 (5)	149 (259)	1 (11)
Final	49 (28)	14 (72)	200 (759)	1 (7)
Median (min–max)				
Baseline	36 (24–118)	2 (<LLOQ–35)	110 (<LLOQ–2332)	0.3 (0.1–114)
Final	42 (14–240)	2 (<LLOQ–695)	110 (<LLOQ–7890)	0.4 (0.1–80)

LLOQ, lower limit of quantification. ^aBaseline values were obtained on or before day 1 of study drug administration and were not available for all patients.

Coppola *et al.* [37], all 11 thrombotic events reported in patients with haemophilia B were of superficial thrombophlebitis, mostly occurring at the infusion site in surgical patients. FVIII activity levels were not collected in the surgical population of this analysis; however, despite any potential increase in FVIII activity levels that may have taken place, no thrombotic events were reported [37]. It should be noted that venous thromboembolism prophylactic strategies undertaken in the surgical population of this analysis were not part of the study analysis and therefore were not collected during the study.

In one retrospective report based on data from a large, commercial insurance database, the same-day rate (per 1000) of thrombotic events in patients receiving clotting factor replacement products was much higher in patients without congenital factor deficiency (70.2 per 1000 patients) than in those with congenital factor deficiency (6.4 per 1000 patients) [38]. The rate of 6.4 thrombotic events per 1000 patients approximates 1.4 events per 221 patients (i.e., the number of patients included in this study). The absence of thrombotic events observed in patients receiving nonacog alfa in this pooled analysis is, therefore, consistent with the low rate of thrombotic events reported in patients with congenital factor deficiency receiving replacement factor products, as reported elsewhere [38].

Coagulation markers were originally derived for predicting the risk of thrombosis or recurrent thrombosis and for guiding anticoagulation therapy [10] in people who have intact coagulation physiology. It is uncertain whether the assessment of F₁₊₂, TAT, and D-dimer levels may help to identify patients with haemophilia B who are at higher risk of thrombosis, in part owing to the limited number of patients in this population, making large prospective trials unfeasible. Although rare cases of peripheral thrombophlebitis and deep vein thrombosis have been reported in haemophilia patients receiving FIX replacement therapy [16], it is not evident that incremental changes in F₁₊₂, TAT, and D-dimer levels would have predicted these events.

Collectively, the clinical and coagulation marker data presented herein raise questions regarding the value of frequent or routine monitoring of F₁₊₂, TAT, and D-dimer in patients with haemophilia B receiving FIX products. In addition to the inconvenience to the patient and the added costs, monitoring for F₁₊₂ requires separate venepunctures [18], increasing the risk of loss of long-term blood vessel patency. Although careful surveillance for potential thromboembolic events remains crucial in the treatment of haemophilia B, the frequency of coagulation marker surveillance required may warrant re-evaluation. The accumulating data suggest that less frequent thrombogenicity monitoring and/or testing based on an individual patient's medical need might

be considered for studies of FIX products. Guidance is also needed regarding the interpretation of changes in coagulation marker levels in individuals with haemophilia B who are receiving FIX products and, if changes are observed, to determine what clinical interventions should be undertaken.

In conclusion, nonacog alfa was not associated with thrombotic risk in paediatric, adult, or surgical patients with haemophilia B who received different treatment regimens or doses, including regular doses of 100 IU/kg. Pooled data from the subset of patients with coagulation marker values showed no evidence of thrombogenicity or increased coagulation activation, as measured by F₁₊₂, D-dimer, and TAT, between baseline levels and final study visits. Routine evaluation of coagulation markers in the context of the haemophilia B clinical studies warrants further discussion among regulatory agencies, the scientific community, and pharmaceutical companies to define appropriate monitoring of the thrombotic risk guidelines.

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Conflicts of interest

All authors are employees of Pfizer Inc and own stock in that company. Medical writing and editorial support were provided by Bina Patel, PharmD, of Peloton Advantage (Parsippany, NJ) and funded by Pfizer Inc.

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