Methods

Study conduct

The trial was approved by independent ethics committees or institutional review boards of all participating sites, and patients or their legal guardians provided written informed consent. The trial was conducted in accordance with the Declaration of Helsinki[10] and good clinical practice.[11]

Patients

Male patients aged 13 to 70 years with hemophilia B (FIX activity ≤2 IU/dL), with no history of inhibitors to FIX, and with at least 150 exposure days to any FIX product were included in the trial. Patients with a history of thromboembolic events (such as myocardial infarction or deep vein thrombosis) and immune-deficient patients with a CD4 lymphocyte count lower than 200 cells/μL were excluded. The complete eligibility criteria are provided in the supplemental Material, Sections 2-4, available on the *Blood* Web site.

Trial design

This was a multinational, randomized (prophylaxis groups only), single-blind trial with 2 nonacog beta pegol prophylaxis groups (10 and 40 IU/kg once weekly) and a single on-demand group. Single-blind meant patients and investigators were blinded to the prophylaxis dose, but the investigator could become unblinded if the FIX activity needed to be measured. The 10 IU/kg and 40 IU/kg once-weekly prophylactic treatment regimens were based on the modeling of pharmacokinetic data from the first human dose trial with nonacog beta pegol in patients with hemophilia B.[8,9] At screening, the patient and investigator decided whether prophylaxis or an on-demand treatment regimen would be used. Patients who chose prophylaxis were randomly assigned 1:1 in a blinded fashion to 1 of the 2 prophylaxis groups. Assessments for safety and efficacy were performed during 10 visits at 4- to 8-week intervals throughout the trial. The duration of the trial was 52 weeks for prophylaxis patients and 28 weeks for on-demand patients. Bleeding episodes were treated with a single dose of 40 IU/kg nonacog beta pegol. If a severe bleeding episode (intracranial, retroperitoneal, iliopsoas, and neck) occurred, it was to be treated with a single dose of 80 IU/kg. The trial product was administered as intravenous bolus injections. A subset of prophylaxis patients was included in the pharmacokinetic assessments with 10 IU/kg and 40 IU/kg nonacog beta pegol.

Outcome measures

The primary safety end point was development of FIX inhibitors analyzed with a Nijmegen modified Bethesda assay during the trial.[12] A patient was considered positive for FIX inhibitors if 2 consecutive samples had a titer of 0.6 Bethesda Units or higher. In addition, all samples were analyzed at the end of the trial in a heat/cold Nijmegen modified FIX Bethesda assay, where the effect of high residual FIX activity was minimized and the sensitivity of the assay increased to low titers of inhibitors (see supplemental Material, Section 6 for details). Other safety assessments included adverse events, assessment of noninhibitory binding antibodies against nonacog beta pegol and antibodies against host cell proteins, and clinical laboratory tests (see supplemental Material, Section 12 for details).

A main efficacy end point was the hemostatic effect of nonacog beta pegol when treating a bleeding episode. Patients classified the bleeds as spontaneous or traumatic and reported the duration of the bleed, the number of doses, and the amount of nonacog beta pegol used to treat the bleed. Patients also rated the hemostatic response from the time of treatment until 8 hours after treatment on a 4-point scale as excellent, good, moderate, or poor (see Table 1 for definitions of hemostatic responses). Bleeding episodes with an excellent or good hemostatic response were considered successful, whereas bleeding episodes with a moderate or poor hemostatic response were considered failures.

Another main efficacy end point was the prophylactic effect of nonacog beta pegol, assessed by estimating the annualized bleeding rates (ABRs) in the trial. An ABR during the last 12 months before the trial was calculated on the basis of the patient-reported number of bleeding episodes during this time.

Table 1. Details of bleeding episodes and hemostatic response

Details	10 IU/kg	40 IU/kg	On demand	All
Number of patients	30	29	15	74
Number of patients with bleeding episodes	25	16	14	55
Number of bleeding episodes	132	70	143	345
Type of bleeding episode, n (%)				
Spontaneous	91 (68.9)	34 (48.6)	102 (71.3)	227 (65.8)
Traumatic	39 (29.5)	36 (51.4)	41 (28.7)	116 (33.6)
After minor surgery	1 (0.8)	—	—	1 (0.3)
Other	1 (0.8)	—	—	1 (0.3)
Injections needed to treat bleeding episode (from start to stop of bleeding episode), n (%)				
1 injection	111 (84.1)	69 (98.6)	120 (83.9)	300 (87.0)
2 injections	16 (12.1)	—	20 (14.0)	36 (10.4)
3 injections	2 (1.5)	1 (1.4)	—	3 (0.9)
≥4 injections	3 (2.3)	—	3 (2.1)	6 (1.7)
Hemostatic response, n (%)*				
Excellent	41 (31.1)	35 (50.0)	43 (30.1)	119 (34.5)
Good	72 (54.5)	32 (45.7)	92 (64.3)	196 (56.8)
Moderate	13 (9.8)	2 (2.9)	7 (4.9)	22 (6.4)
Poor	4 (3.0)	—	—	4 (1.2)
Missing	2 (1.5)	1 (1.4)	1 (0.7)	4 (1.2)
Successful hemostatic responses, n (%)†,¶	113 (86.9)	67 (97.1)	135 (95.1)	315 (92.4)
Failure hemostatic responses, n (%)‡,¶	17 (13.1)	2 (2.9)	7 (4.9)	26 (7.6)

*Definitions of hemostatic response: excellent = abrupt pain relief and/or clear improvement in objective signs of bleeding within 8 hours after a single injection; good = noticeable pain relief and/or improvement in signs of bleeding within 8 hours after a single injection; moderate = probable or slight beneficial effect within the first 8 hours after the first injection but requiring more than a single injection within 8 hours; poor = either no improvement or worsening of symptoms within 8 hours after 2 injections.

†A successful hemostatic response was defined as an excellent or good hemostatic response. Note that this observed rate is slightly different from the model-based estimate presented in Results, which was 92.2%.

‡A failure hemostatic response was defined as a moderate or poor hemostatic response.

¶Bleeding episodes with a missing hemostatic response were not included in the calculation of successful and failure hemostatic responses.

Predose FIX trough activities were estimated and compared statistically with a trough activity of 1 IU/dL. Emphasis was on the resolution of target joints (a target joint was defined as 3 or more bleeding episodes in a particular joint within a period of 6 months before trial). For *F9* genotyping, null mutations included large deletions/insertions, inversions, and nonsense mutations. Nonnull mutations included small deletions/insertions, splice site and missense mutations, and substitutions.[13] The presence and extent of arthropathy in joints at baseline was recorded at the discretion of the investigator and was based on descriptions by the patients.

Pharmacokinetic assessments were performed at trial initiation (single dose assessments) and after 12 to 44 weeks of prophylaxis (steady state assessments) with 10 IU/kg and 40 IU/kg. The assessments included 7 sampling points up to 168 hours postinjection. Pharmacokinetic assessments of FIX activity were based on a 1-stage clotting assay performed at a central laboratory and using a product-specific reference standard for calculation of the FIX activity (see supplemental Material, Section 9 for details).

Patient-reported outcomes were collected from all patients using the EuroQoL-5 Dimensions (EQ-5D) visual analog scale (VAS).

Statistical methods

The analyses are based on all patients exposed to nonacog beta pegol. With respect to the primary outcome measure (incidence of patients with FIX inhibitor), no formal sample size calculation was performed because of the

rarity of the disease. A sample size of 50 patients was considered sufficient for a reasonable evaluation of FIX inhibitor formation. The incidence rate of inhibitors is reported with a 1-sided 97.5% upper confidence limit, based on an exact calculation for a binomial distribution. Adequate safety with regard to FIX inhibitors is concluded if the observed rate is lower than or equal to 2% and the upper 1-sided 97.5% confidence limit is below or equal to 10.7%.

Secondary outcome measures included the hemostatic effect, the ABR, FIX trough activity levels, pharmacokinetic analyses, assessment of target joints, and health-related quality-of-life (HR-QoL) measures.

With respect to estimating the success rate when treating bleeding episodes, a logistic regression with a repeated statement to account for correlation within patients was used, applying an exchangeable working correlation matrix. This model specified that multiple bleeding episodes for a particular patient were not treated as independent events and assumed the same within-patient correlation for all bleeding episodes. A hemostatic effect of nonacog beta pegol was concluded if the lower 95% confidence limit for the success rate (success defined as excellent or good hemostatic response) was above 65%. The observed success rate was calculated as the number of successfully treated bleeds divided by the total number of treated bleeds.

Key measures of the effect of prophylaxis were the median of the individual ABRs and the estimated mean ABR. These were estimated from a Poisson regression model with dose as factor, allowing for overdispersion and using treatment duration as offset. This allowed for ABRs to be estimated for each treatment regimen group while accounting for various durations of treatment. Individual ABRs were calculated as the number of bleeding episodes per patient scaled to a treatment duration of 1 year. Prophylactic effect of nonacog beta pegol was concluded if the upper 95% confidence limit for the ABR was below 4.8, which corresponds to a more than 60% reduction of a literature-based expected ABR for on-demand patients of 12 bleeding episodes/patient/year.[14-17] This requirement for claiming prophylactic effect proved to be a conservative measure compared with the actual on-demand ABRs in the trial.

Efficacy of nonacog beta pegol was also assessed by FIX trough activity as a surrogate marker. Demonstration of an estimated mean FIX trough level significantly above 1 IU/dL was performed using a mixed model analysis of the log-transformed FIX activity, with dose as a factor and patient as a random effect. Only samples collected between 5 and 10 days after last dose and at least 14 days after last bleeding episode were included.

The number of target joints per patient was collected at baseline, and the number of bleeding episodes in target joints during the trial was summarized by treatment group.

Pharmacokinetic parameters were calculated using standard noncompartmental methods (see supplemental Material, Section 11 for definition and calculation of pharmacokinetic parameters).

HR-QoL was assessed using the EQ-5D VAS instrument and presented descriptively.

Drug product

Nonacog beta pegol was produced by expression in Chinese hamster ovary cells and was supplied as freeze-dried powder in single-use vials with a nominal content of 500 IU/vial or 2000 IU/vial. Both strengths were reconstituted with 4.2 mL histidine solvent for intravenous injection.

Results

Patients

From April 2011 through April 2013, 74 patients with hemophilia B (FIX ≤2 IU/dL) were enrolled and dosed at 39 sites in 13 countries (supplemental Material, Section 13). Of these, 67 patients (17 adolescents [13-17 years] and 50 adults [18-65 years]) completed the trial (Figure 1). None of the 7 withdrawals (2 patients in the 10 IU/kg prophylaxis group, 3 patients in the 40 IU/kg prophylaxis group, and 2 patients in the on-demand treatment group) were a result of adverse
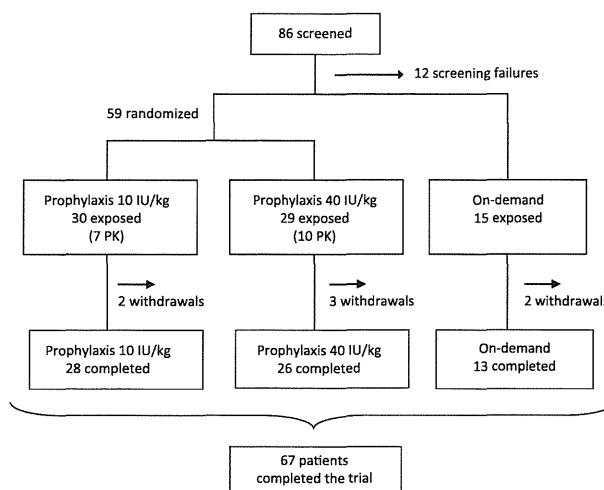

Figure 1. Patient enrolment and outcomes. A total of 86 patients were screened, of whom 12 were screening failures, leaving 74 patients who were exposed to nonacog beta pegol. At the screening visit, the patient and the investigator decided together whether the patient should be allocated to prophylaxis (59 patients) or on-demand treatment (15 patients). Patients allocated to prophylaxis were randomly assigned to once-weekly dosing of either 10 or 40 IU/kg. A total of 7 patients were withdrawn during the trial, distributed evenly between the treatment groups. None of the withdrawals were a result of adverse events. Screening failures and withdrawals together constituted 19 (22%) of the 86 screened patients. A total of 17 patients participated in a pharmacokinetic session at trial initiation, and all but 1 had a second pharmacokinetic session after 12 to 44 weeks of prophylaxis, leaving 16 patients (7 in the 10 IU/kg group and 9 in the 40 IU/kg group) with complete pharmacokinetic assessments.

events (see supplemental Material, Section 5 for details on withdrawals). Patient characteristics are presented in Table 2.

Safety

Mean number of exposure days to nonacog beta pegol was 54 for patients receiving prophylaxis and 14 for patients receiving on-demand treatment. No patients developed FIX inhibitors, and no deaths, thromboembolic events, or allergic reactions related to nonacog beta pegol occurred. One patient was positive for host cell protein antibodies before and after exposure. Two patients in the 10 IU/kg group and 1 patient in the 40 IU/kg group were transiently positive for noninhibitory FIX-binding antibodies (2 were positive before exposure). The highest antibody titer for these 3 patients was 4; it did not affect drug recovery and had no effect on bleeding patterns. None of the antibodies were present at the end of trial (see supplemental Material, Section 10 for details).

A total of 215 adverse events (7 severe, 25 moderate, and 183 mild) in 60 (81%) patients were reported, corresponding to 3.33 adverse events per patient year of exposure. The most commonly reported adverse events were nasopharyngitis (13 events in 10 patients [13.5%]), influenza (10 events in 8 patients [10.8%]), and upper respiratory tract infection (10 events in 8 patients [10.8%]), which are not unusual observations in clinical trials. There were 4 serious adverse events (hip fracture, worsening of skin ulcer, retroperitoneal hematoma, and abdominal pain) in 4 patients (5.4%). These serious adverse events were reported by the investigator as unlikely to be related to nonacog beta pegol. No safety concerns were identified from physical examinations or clinical laboratory tests.

Efficacy

Treatment of bleeding episodes. A total of 345 bleeding episodes in 55 (74%) patients were treated with nonacog beta pegol, of which 202 bleeding episodes were in prophylaxis patients and 143 bleeding

Table 2. Baseline demographics and patient characteristics

Characteristics	10 IU/kg	40 IU/kg	On demand	All
Number of patients	30	29	15	74
Age, years				
Mean (SD)	32.4 (13.9)	30.0 (15.8)	32.4 (12.0)	31.4 (14.2)
Weight, kg				
Mean (SD)	75.6 (13.0)	70.4 (15.1)	76.1 (16.6)	73.7 (14.7)
Race, n (%)				
White	16 (53.3)	21 (72.4)	11 (73.3)	48 (64.9)
Asian	8 (26.7)	5 (17.2)	3 (20.0)	16 (21.6)
Black or African American	2 (6.7)	3 (10.3)	—	5 (6.8)
Other	4 (13.3)	—	1 (6.7)	5 (6.8)
Previous treatment regimen,* n (%)				
Prophylaxis	20 (66.7)	17 (58.6)	2 (13.3)	39 (52.7)
On demand	10 (33.3)	12 (41.4)	13 (86.7)	35 (47.3)
Previous prophylaxis patients, n (%)				
Recombinant FIX	11 (55.0)	10 (58.8)	—	21 (53.8)
Plasma FIX product	9 (45.0)	7 (41.2)	2 (100.0)	18 (46.2)
Classification of hemophilia,† n (%)				
Moderate (1-2 IU/dL)	7 (23.3)	5 (17.2)	2 (13.3)	14 (18.9)
Severe (<1 IU/dL)	23 (76.7)	24 (82.8)	13 (86.7)	60 (81.1)
F9 genotype,‡ n (%)				
N	27 (100.0)	24 (100.0)	15 (100.0)	66 (100.0)
Null mutations	7 (25.9)	1 (4.2)	5 (33.3)	13 (19.7)
Nonnull mutations	20 (74.1)	23 (95.8)	10 (66.7)	53 (80.3)
Arthropathy at baseline,¶ n (%)				
Yes	20 (66.7)	18 (62.1)	10 (66.7)	48 (64.9)
No	10 (33.3)	11 (37.9)	5 (33.3)	26 (35.1)
Target joints at baseline,§ n (%)				
Yes	13 (43.3)	15 (51.7)	12 (80.0)	40 (54.1)
No	17 (56.7)	14 (48.3)	3 (20.0)	34 (45.9)

FIX, coagulation factor IX; SD, standard deviation.

*Two patients receiving previous prophylaxis chose to enroll into the on-demand treatment group. These 2 patients entered the trial at a time at which the prophylaxis groups were not yet opened for enrollment because of regulatory authority requirements in the patients' country of origin.

†Classification as defined in medical records. Only patients with FIX activity 2 IU/dL or less were eligible.

‡Null mutation genotypes included large deletions/insertions, inversions, and nonsense mutations. Nonnull mutations included small deletions/insertions, splice site and missense mutations, and substitutions.[13] Mutations in the F9 gene were determined by either laboratory analysis carried out in the trial or, alternatively, by post hoc classification of gene defects reported in patients' medical records where possible.

¶The presence and extent of arthropathy in joints at baseline was recorded at the discretion of the investigator and was based on descriptions by the patients.

§A target joint was defined as 3 or more bleeding episodes in a particular joint within a period of 6 months before trial.

episodes were in on-demand patients. Approximately two-thirds were reported as spontaneous and one-third as traumatic (Table 1). The majority of bleeding episodes (78.5%) were in joints (see supplemental Material, Section 15). The overall success rate for treatment of all bleeding episodes was estimated to be 92.2% (95% confidence interval [CI], 86.9-95.4). In the 40 IU/kg group, 99% of the bleeding episodes were resolved with 1 injection of nonacog beta pegol compared with 84% in both the 10 IU/kg and on-demand groups (Table 1). One severe bleeding episode was reported in the trial: a bleeding episode in the knee joint in a patient in the 10 IU/kg prophylaxis group. The bleeding episode was treated with 80 IU/kg nonacog beta pegol, and the hemostatic response was rated as excellent.

Prophylaxis. The number of patients who did not have a treatment-requiring bleeding episode during the trial was 5 (17%) of 30 patients in the 10 IU/kg group, 13 (45%) of 29 patients in the 40 IU/kg group, and 1 (7%) of 15 patients in the on-demand group. The median ABR and the estimated mean ABR in the 10 IU/kg group were 2.93 (interquartile range [IQR], 0.99-6.02) and 4.56 (95% CI, 3.01-6.90), respectively. The median ABR and the estimated mean ABR in the 40 IU/kg group were 1.04 (IQR, 0.00-4.00) and 2.51 (95% CI, 1.42-4.43), respectively (Table 3).

Furthermore, 10 (66.7%) of 15 patients with target joints at trial entry had no treatment-requiring bleeding episodes in their target joints during prophylaxis with 40 IU/kg treatment compared with 1 (7.7%) of 13 patients in the 10 IU/kg group. In the on-demand group, 2 (16.7%) of 12 patients did not bleed into their target joints during the trial. Both of these on-demand patients entered the trial from a previous on-demand treatment regimen, whereas 1 of the patients withdrew for personal reasons after 2.5 months in the trial.

ABRs estimated from spontaneous bleeding episodes occurring up until and including 4 days from previous prophylactic treatment, or later than 4 days from previous prophylactic treatment, were 1.87 (95% CI, 1.08-3.23) and 2.16 (95% CI, 1.21-3.88), respectively. Thus, bleeding rates were only slightly higher toward the end of the dosing intervals, reflecting that patients had high FIX activity levels during the entire time between prophylactic dose administrations. Reduction in ABR for those patients who entered the trial from a prophylaxis treatment regimen was most pronounced in the 40 IU/kg group (from 7.49 to 3.33) compared with in the 10 IU/kg group (from 5.13 to 4.68) (Table 3). The frequencies of bleeding episodes in patients are presented in the supplemental Material, Section 16.

FIX trough activity

Throughout the trial, the mean predose FIX activities were statistically significantly above 1 IU/dL for both the 10 IU/kg and the 40 IU/kg prophylaxis groups (Figure 2). The estimated mean FIX trough activities were 8.5 IU/dL (95% CI, 7.7-9.3; $P < .001$) and 27.3 IU/dL (95% CI, 24.8-30.0; $P < .001$), respectively.

Pharmacokinetics

The single-dose and steady state geometric mean half-lives of nonacog beta pegol were 93 hours (coefficient of variation [CV%] 19.5) and 107 hours (CV% 21.8), respectively, in the 10 IU/kg group, and 85 hours (CV% 21.8) and 111 hours (CV% 11.8), respectively, in the 40 IU/kg group. Incremental recovery (between 0.02 and 0.03 [IU/mL]/[IU/kg]) and clearance (between 0.36 and 0.43 mL/h/kg) were similar between the 2 dose groups (see supplemental Material, Section 17).

Patient-reported outcomes

HR-QoL was measured on the EQ-5D VAS scale. In the 10 IU/kg group, the median score was 80 (range, 30-100) at trial entry and 85 (range, 0-100) at the end of the trial. In the 40 IU/kg group, the median score increased from 75 (range, 35-100) at trial entry to 90 (range, 60-100) at trial end (see supplemental Material, Section 14 for details).

Discussion

This prospective trial demonstrated that nonacog beta pegol was safe and effective for the treatment and prevention of bleeding episodes in patients with FIX deficiency. No clinically significant safety issues

Table 3. ABRs

Characteristics	10 IU/kg	40 IU/kg	On demand
All patients			
N	30	29	15
Median (IQR)	2.93 (0.99-6.02)	1.04 (0.00-4.00)	15.58 (9.56-26.47)
Estimated rate (95% CI)*	4.56 (3.01-6.90)	2.51 (1.42-4.43)	—
P value†	.40	.01	
Previous prophylaxis patients			
N	20	17	2
Bleeding rate during the last 12 months before trial‡			
Median	4.75	4.00	9.50
Estimated rate	5.13	7.49	9.50
Bleeding rates during trial			
Median	2.99	1.93	25.69
Estimated rate	4.68	3.33	29.4
Previous on-demand patients			
N	10	12	13
Bleeding rate during the last 12 months before trial‡			
Median	14.0	12.5	15.0
Estimated rate	17.9	21.2	22.7
Bleeding rates during trial			
Median	2.06	0.52	13.0
Estimated rate	4.30	1.32	17.6
All patients by type of bleed			
Spontaneous bleeding episodes			
Median (IQR)	0.97 (0.00-4.01)	0.00 (0.00-0.98)	11.1 (7.16-15.8)
Estimated rate (95% CI)*	3.14 (1.78-5.56)	1.22 (0.48-3.10)	
Traumatic bleeding episodes			
Median (IQR)	0.98 (0.00-1.93)	0.00 (0.00-2.04)	1.73 (0.00-8.95)
Estimated rate (95% CI)*	1.35 (0.81-2.24)	1.29 (0.76-2.19)	

*Estimated rates for prophylaxis patients are based on a Poisson regression model with dose as a factor, allowing for overdispersion and using treatment duration as an offset.

†P values are from the 1-sided test of the null hypothesis that the estimated rate is at least 4.8, evaluated at the 2.5% level.

‡Bleeding rate during the last 12 months before trial on that particular treatment regimen.

were identified in the trial. Specifically, no FIX inhibitors developed and no thrombotic or hypersensitivity events related to treatment with nonacog beta pegol were reported.

Once-weekly injections with nonacog beta pegol resulted in substantially higher FIX trough activities compared with currently available standard recombinant FIX products, reflecting that patients had high FIX activity levels during the entire time between the nonacog beta pegol prophylactic dose administrations.[6,18-21] A recently approved long-acting FIX product, rFIXFc, demonstrated a terminal half-life of 82.1 hours when patients were dosed 50 IU/kg once weekly, whereas the steady state half-life of nonacog beta pegol was between 107 and 111 hours.[21] However, key differences between the trials for rFIXFc and nonacog beta pegol, namely prophylaxis regimes, dose adjustments, and pharmacokinetic analysis, make direct comparisons between these products

difficult.[21] With the prolonged half-life and high incremental recovery of nonacog beta pegol, patients with severe or moderate hemophilia B should be able to exhibit a bleeding phenotype of mild hemophilia B with FIX activity well above 5 IU/dL.[1]

In the 40 IU/kg prophylaxis group, an improvement in patient-reported HR-QoL VAS score from a median of 75 at trial entry to 90 after 52 weeks of treatment was observed. This change during the trial, moving the patients in the 40 IU/kg group to a score similar to that of a general population,[22] was likely related to fewer bleeding episodes, fewer injections, resolution of target joint bleeding, or a combination of these and other factors.

The success rate for treatment of all bleeding episodes was estimated to be 92.2% (95% CI, 86.9%-95.4%), demonstrating a hemostatic effect of nonacog beta pegol in line with currently available FIX products.[6,18,20]
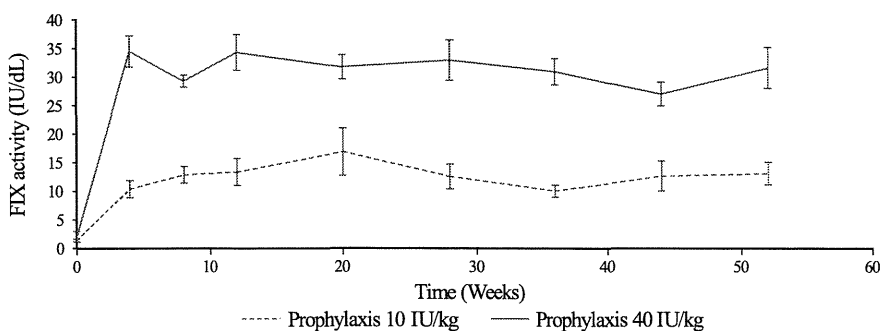

Figure 2. Mean predose FIX activity during trial. The graph shows the mean (± standard error of the mean) FIX activity in predose samples for the 10 IU/kg and 40 IU/kg prophylaxis groups. A 1-stage clotting assay was used to determine the FIX activity (IU/dL) in predose blood samples collected at clinical visits during the trial. Analysis was performed at a central laboratory, using a product-specific reference standard for calculation of the FIX activity.

The observed bleeding rates in both the 10 IU/kg and 40 IU/kg prophylaxis groups were similar to, or better than, reported bleeding rates for commercially available products.[18,20,21,23] The predefined criteria for effective prophylaxis (estimated mean ABR significantly below 4.8) were met with the 40 IU/kg dose, but not the 10 IU/kg dose, despite a clinical effect with both doses. In the 40 IU/kg group, 45% of the patients did not bleed at all; this proportion was 17% in the 10 IU/kg group.

Differences between the 2 prophylaxis groups are, however, noteworthy and could be attributed to the substantially higher FIX trough levels observed in the high-dose prophylaxis group. First, patients in the 40 IU/kg group had lower bleeding rates compared with those in the 10 IU/kg group and demonstrated the largest decrease compared with the patients' own bleeding rate during the last 12 months before the trial. Second, when patients receiving prophylaxis bled, those in the higher-dose group were more likely to respond to 1 treatment dose compared with patients in the lower-dose group (99% vs 84%). Third, and perhaps most noteworthy from a clinical perspective, patients with target joints at trial entry had a substantially higher likelihood of resolving their target joint if they were in the higher-dose group, with a 67% resolution rate compared with 7.7% in the lower-dose group.

With the trough levels of FIX activity observed in the prophylaxis groups, spontaneous bleeding episodes may not have been expected, yet approximately 70% and 50% of the bleeding episodes in the 10 IU/kg and 40 IU/kg prophylaxis groups, respectively, were reported by the patients as spontaneous. One explanation could be that the majority of the patients had hemophilic arthropathy in 1 or more joints, such that even in the presence of the attained FIX activity, spontaneous bleeding episodes could occur. Another explanation could be that symptoms of hemophilic arthropathy, such as pain, swelling, and stiffness, may have been perceived as bleeding episodes, and therefore treated as such, even though no bleeding episode had actually occurred.[24] The results presented here demonstrate wide interpatient variability, and this unexpected finding requires further investigation.

Limitations of this trial were the exclusion of patients older than 70 years and those with a history of thromboembolic events, such as myocardial infarction or deep vein thrombosis. The patients' free choice between entering into prophylaxis or receiving on-demand treatment made direct comparisons between the prophylaxis groups and the on-demand group unfeasible. Trial product-related safety assessments in on-demand patients were limited by the few exposure days to nonacog beta pegol during the trial. However, 12 of the 13 on-demand patients who completed the trial chose to progress into the extension trial (ClinicalTrials.gov, #NCT01395810) and have therefore received subsequent doses of nonacog beta pegol beyond the limited exposure in this trial.

In conclusion, no clinically significant safety concerns were identified and no inhibitory antibodies were observed. Nonacog beta pegol was effective for the treatment of bleeding episodes in both prophylaxis and on-demand patients. Low bleeding rates were observed in patients receiving prophylaxis and are likely related to high trough levels resulting from the extended half-life of nonacog beta pegol. Clinical improvement of target joints and HR-QoL was observed in the 40 IU/kg group. These data suggest that once-weekly prophylaxis with nonacog beta pegol may provide a new and safe alternative for the prevention and treatment of bleeding episodes in patients with hemophilia B. Further evaluations in a subsequent extension trial (ClinicalTrials.gov, #NCT01395810) of these patients will hopefully confirm these findings, along with the investigation of nonacog beta pegol in previously treated and previously untreated pediatric patients with hemophilia B (ClinicalTrials.gov, #NCT01467427 and #NCT02141074, respectively). In addition, nonacog beta pegol was investigated in a recently completed clinical trial of patients with hemophilia B undergoing surgery (ClinicalTrials.gov, #NCT01386528).

Authorship

Contribution: P.W.C., G.Y., F.A.K., P.A., T.G., J.M., T.M., E.P.M.-B., J.O., C.E.W., and C.N. were principal investigators and enrolled and cared for patients during the trial. K.K. and C.B. were involved in the design of the trial. The authors designed the trial protocol, directed the data analysis, and wrote the manuscript. The sponsor was responsible for trial operations, including data analysis. All authors had access to the primary clinical trial data. All authors were involved in interpretation of the trial results and preparation of the manuscript outline, provided input during the review stages, and approved the final manuscript. The principal investigator (P.W.C.) assumes full responsibility for the accuracy and completeness of the reported data.

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A complete list of the principle paradigm 2 Investigators appears in the online data supplement.

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消費性凝固障害

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▶ 播種性血管内凝固（DIC）における凝固活性化は組織因子（tissue factor：TF）によるが，トロンビン形成の増幅は生理的な抗凝固システムの機能不全も関与する．

▶ 血小板・血漿による補充療法は RCT に基づくエビデンスはなく，血漿凝固因子レベルや血小板数の補正のみが消費性凝固障害治療の中心ではない．

▶ アンチトロンビン（antithrombin：AT），活性化プロテイン C（activated protein C：APC），トロンボモジュリン（thrombomodulin：TM）などナチュラルな抗凝固因子の投与によりバランスを改善することも消費性凝固障害の治療戦略の一つである．

　DIC において凝固システムの全身的な活性化はさまざまな基礎疾患に伴って発生します．活性化を示す分子マーカーが利用されるようになり，臨床現場における有用性も検証されつつありますが，臨床症状としては，DIC における凝固の活性化は血小板の低下に始まり，いわゆる global clotting time（PT，ときに APTT）の延長に至ることで認識されます．

　生理的条件下では，ヒトの凝固システムではクロット（凝血塊）形成を促進する方向と抑制する方向（抗凝固システム）が絶妙なバランスで成り立っており，DIC や重篤な肝障害の状態においてすら，しばしば微妙なバランスを保っていますが，これらの病態におけるバランスは，所詮不安定であり，容易に壊れやすいです（図 1）．

DIC における凝固活性化のメカニズムについて教えてください

　DIC における凝固活性化は TF によりますが，トロンビン形成の増幅は生理的な抗凝固システムの欠如によることが大きいと最近では定義されるようになってきました．微小環境における広範なフィブリン形成に線溶システムが追いつかず，一部の病態においては線溶システムそのものの機能不全も寄与しています（図 1）．

図1 Simplified model of hemostasis equilibrium
(A) 生理的環境
(B) DIC における止血の均衡性. 血漿中凝固因子は最低必要量近くまで低下しているが, 線溶因子・凝固因子も同様に低下している場合はバランスがとれ, 症状も発現は少ない. ただし, 双方とも減少しているため, 容易にバランスが崩れやすい.
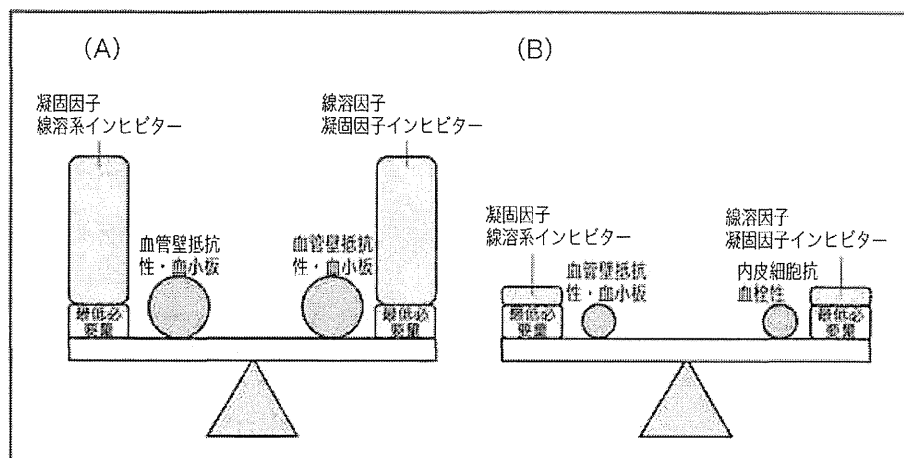
組織因子 (TF)

　TF は, 基本的かつ生理的な凝固反応開始の initiator であり, また多くの血栓性疾患の発症において病的にも重要です. TF はさまざまなタイプの細胞上に存在する膜蛋白であり, 血液と接することにより血漿中の酵素前駆体 (zymogen) の第VII因子 (Factor VII, 以下 FVII), もしくは加水限定分解により酵素学的に active なフォームとなった活性型第VII因子(FVIIa)と強く結合します. TF は FVIIa に対してアロステリックなレギュレーター(またはコファクター) として働くと考えられており, FVIIa の酵素活性を大幅に上昇させます[1,2]. TF-FVIIa 複合体の天然基質はセリン酵素前駆体である第IX因子 (FIX), 第X因子 (FX) であり, 活性化されたこれらの凝固因子がトロンビンを産生, フィブリノゲンをフィブリンに凝固させ, また血小板を活性化させます.

　DIC における TF の関与は以前から指摘されており[3,4], また数多くの実験結果からエンドトキシン血症などによる DIC において, 単球が重要な役割を示すであろうことが推測されています.

　TF は多くのがん細胞株やがん組織に発現しており[5], 特に血液悪性腫瘍においては TF 活性, 抗原, あるいは mRNA 量の増加が急性骨髄性白血病 (AML), 特に急性前骨髄球性白血病 (APL) や, 一部の急性リンパ性白血病 (ALL) においてもみられます[5,6]. これらの腫瘍組織における TF の発現は DIC の合併に密接に関係があり, APL においては単球や[7] 白血病細胞そのもの[8] を all-trans-retinoicacid (ATRA) で処理すると, TF 産生が抑制されるという実験結果から, ATRA が腫瘍細胞に直接働いて, 全身的な凝固活性化を抑制する可能性も示唆されています[9].

❓ 抗凝固系による凝固の制御について教えてください

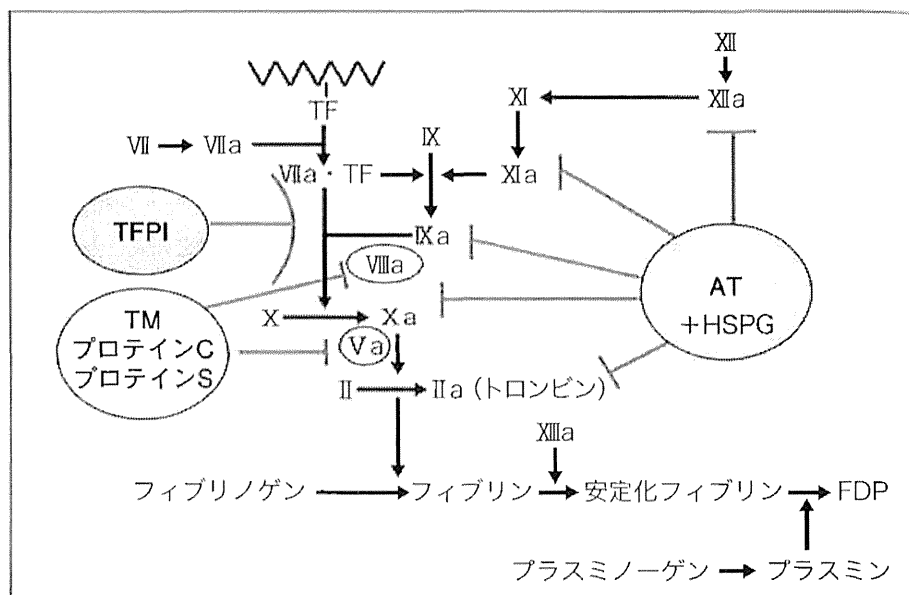　凝固系の全身的な活性化は生理的な coagulation inhibitor (抗凝固因子) によってバランスが保たれます. このシステムは大きく分け

図2　抗凝固因子による制御

て，内皮細胞ヘパラン硫酸＋血漿中 AT による系，TM，プロテイン C（PC）とプロテイン S（PS）による系，tissue factor pathway inhibitor（TFPI）が含まれます．

　図2に，本稿で取り上げる抗凝固因子について，その相互関係を示しました．アンチトロンビン（AT，AT Ⅲ）はトロンビン，活性化凝固第 X 因子などに対する生理的阻害因子であり，おそらく血液凝固制御においてもっとも重要な役割を果たします．ただし，その阻害活性はヘパリン，生体内においてはヘパラン硫酸プロテオグリカン（HSPG）の存在下に著しく促進されます．一方，PC は血管内皮細胞上のトロンビン–TM 複合体により活性化され，PS を補酵素として活性化凝固第 V 因子（FVa）および第Ⅷ因子（FⅧa）を阻害します．

■ アンチトロンビン（AT）

　AT は，主に肝臓で産生される 58 kDa の 1 本鎖の糖蛋白であり，セリンプロテアーゼを阻害するセルピンの代表的分子です．主として，セリンプロテアーゼであるトロンビンを不活化しますが，活性化第 X 因子（FXa），第Ⅸ因子（FⅨa）をも不活化します．AT の抗トロンビン活性は，先述したようにヘパリン，ヘパラン硫酸の存在が不可欠です．

　AT 欠損型マウスによる検討[10]では，妊娠 16.5 日以後は胎性致死となります．死亡した AT 欠損型胎児は，全身性の皮下出血をきたしていました．これら死亡した AT 欠損型胎児を組織学的に検討した結果，心臓と肝臓に広範なフィブリンの沈着を認めました[10]．また，肝臓では中心静脈にはフィブリンの沈着を認めず，類洞に沿ってフィブリンの沈着を認めました．死亡した AT 欠損型胎児における皮下出血，頭蓋内や腹腔内にも出血が認められましたが，これらの部位ではフィブリンの沈着を免疫染色では認めず，AT 欠損型胎児では心筋と肝臓における広範なフィブリン沈着により

血液凝固因子が欠乏した状態，いわゆる消費性凝固障害が生じていたことが示唆されました．

DICでは，トロンビンやそのほかの凝固因子の生成亢進に伴い，血中AT活性は低下しますが，重症感染症などに伴うDICで認められる血中AT活性低下は，むしろ炎症（血管外漏出）もしくは肝の蛋白合成能低下によると考えられます．敗血症の病態形成には炎症性サイトカインが重要な役割を演じるので，AT活性の低下の程度が大きいほど予後が不良であることが示されています．また血中AT濃度の上昇・維持はDICの治療においても重要であることが示されており[11]，AT活性測定は，AT製剤補充の適応を決めるうえで重要な検査です．ただ，肝障害でも肝合成能低下により血中AT活性が低下するので，肝不全に重症感染症が合併すると，さらに血中AT活性は低下します．ネフローゼ症候群や熱傷では，それぞれ，尿中および血管外への漏出により血中AT活性が低下し，大手術後，L-アスパラギナーゼ投与によっても低下することが知られています．

▌ トロンボモジュリン（TM）とプロテインC（PC）

TMは，主に血管内皮細胞に発現している抗凝固因子であり，血中に出現するトロンビンと1：1複合体を形成して凝固阻害因子のPCを活性化します．TMはその名のごとく，強力な凝固促進酵素であるトロンビンを（PCの活性化という意味で）抗凝固酵素に瞬時に切り替えることができ，ほ乳類の抗凝固システムにおいてもっとも精密に発展した機能を発現すると考えられます（図3）．TMはほとんどすべての臓器の動静脈，毛細血管，リンパ管，血小板などに分布します．変異TMを用いた研究から，TMのコファクター活性の発現には第4〜6EGF様ドメインが必要です．一方，可溶性TMは，その遺伝子組み換え品が世界に先駆けてDICに対して認可された天然型抗凝固因子となり，その有用性が注目されています[12]．

▌ プロテインC（PC）

第X因子，第IX因子，第VII因子などと同様に，ビタミンK依存性に肝

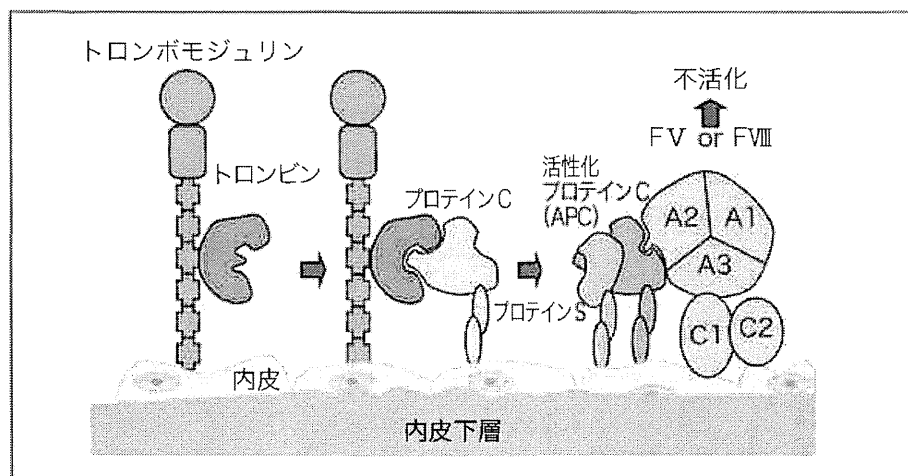**図3　TM-プロテインCによる凝固制御系**
A1, A2, A3, C1, C2はFV，FVIIIのドメイン．

臓で合成されます．ヒトPCの分子量は約6万2,000であり，循環血液中の一部（10～15％）は1本鎖分子ですが，大部分はS-S結合で連結された2本鎖〔軽（L）鎖（分子量2万1,000）および重（H）鎖（分子量4万1,000）〕分子として存在します．セリンプロテアーゼ前駆体であるPCは，内皮細胞上のTMに結合したトロンビンによって活性化されます．APCは，血小板や血管内皮細胞膜のリン脂質にCa²⁺の結合したGlaドメインを介して結合し，PSおよびFVの存在下に活性型凝固補酵素蛋白質のFⅧaおよびFVaを分解・失活化します．

一方，内皮細胞上のPCレセプターであるEPCRは，トロンビン-TM複合体に基質PCを提示することにより，PC活性化の反応を高めます．PCの先天性欠損症のホモ接合体は電撃性紫斑病とよばれ，新生児において消費性凝固障害を伴った重篤な血栓塞栓症となります[13]．

消費性凝固障害の臨床症状について教えてください

重症DIC患者においては，臓器症状としての血栓症状を示す一方で，重症出血症状が主症状であることがあります．興味深いことに，古典的なWaterhouse-Friderichsen症候群（汎発性の髄膜炎菌による敗血症DIC）などを除いては，血栓症状と出血症状を両方明らかに呈する患者は多くありません．

DICは疾患としてではなく，あくまでも基礎疾患に伴う合併症としてとらえられるべきでありますから，凝固システムへの介入は必要であるものの，あくまでもサポートと考えられるべきでしょう．

消費性凝固障害の検査所見（PT，APTT以外）について教えてください

▌ FDP，Dダイマー

フィブリン分解産物（fibrin degradation products：FDP）は，フィブリノゲンと凝固系の最終産物であるフィブリンがプラスミンの働きにより，線溶により生じる分解物質の総称です．線溶現象のうち，一次線溶では，フィブリノゲンがプラスミンにより，最終的にD分画とE分画（FDP-E）に分解されます．これらのD・E分画ほかを，フィブリノゲン分解産物（fibrinogen degradation products：FgDP）とよぶことがあります．これに対し，二次線溶では，第ⅩⅢ因子によって架橋された最終産物の安定化フィブリンが，プラスミンによりさまざまな高分子中間産物を経て，最終的にDダイマーとE分画になります．

前記の一次線溶と二次線溶の分解産物は，どちらも抗フィブリノゲン抗体と反応するので，これらすべてが「FDP」ということになります．これらのうち，抗E分画抗体と反応するものをFDP-Eとよび，測定可能です．

*In vitro*におけるフィブリノゲンのプラスミンによる分解速度は，安定

化フィブリンの分解速度に対してかなり遅いことから，血栓溶解療法中や APL などの病態を除いて，通常 FDP の大部分は安定化フィブリンからの分解産物を反映していると考えられ，異常値を示した場合は，二次線溶亢進状態と推定できます．この場合，D ダイマーも上昇しています．

一方，血栓溶解療法中や APL，腹部大動脈瘤，一部の固形がん，重症肝障害などの病態においては，一次線溶亢進状態が主体となっており，FgDP による FDP 上昇の状態が考えられます．これらの場合，FDP 増加に比し D ダイマーの増加率が少なくなり，FDP 濃度が D ダイマー濃度を数倍に上回る乖離が起きることがあります．このような場合には，一次線溶亢進による凝固因子消費がより早く進行しており，出血症状に留意する必要があります．

■ プラスミン・α_2 プラスミンインヒビター複合体（plasmin-α_2 plasmin inhibitor complex：PIC）

線溶状態を把握するには，最終的に形成されるプラスミンを直接測定できればよいですが，プラスミンの半減期は非常に短く，直接測定することは困難です．PIC はプラスミン形成を，より直接的に反映する物質であり，生体内線溶活性化状態を知ることができます．線溶優位型 DIC では，凝固・線溶両者の活性化が著しく，血中 TAT，PIC はいずれも上昇し，α_2PI は著減します（典型例では 50% 未満）．

■ α_2 プラスミンインヒビター（α_2 plasmin inhibitor：α_2PI）

DIC では，凝固系の活性化に引き続く二次線溶反応の亢進により，プラスミノゲン α_2PI 値が，線溶系亢進状態による消費亢進で低下します．プラスミン産生に対して α_2PI の中和により両者の複合体（PIC）が出現することで，フリーの α_2PI が低下します．特に，APL に合併した DIC では，PIC が著増し α_2PI の低下を示すことが知られています．APL においては TF の産生自体も高度ですが，細胞表面上に過剰に発現する Annexin II が tPA によるプラスミン生成を促進する[14]ため，循環中のプラスミン活性が上昇し，フィブリンのみならずフィブリノゲンの分解が起こり，FgDP が産生される結果，FDP の濃度が上昇します．全身的なプラスミン活性の上昇は α_2PI の低下を伴い，線溶亢進のマーカーとして利用価値が高いです．

血漿因子，血小板による補充療法について教えてください

血小板・血漿による補充療法は，RCT に基づくエビデンスはなく，唯一の DIC に対する補充療法の RCT は新生児に対して行われたもので，交換輸血と新鮮凍結血漿（FFP）輸注を比較したものであり[15]，両者に差はありませんでした．確かに血漿凝固因子レベルや血小板数の補正

のみが消費性凝固障害治療の中心ではなく，患者個々の出血症状や，観血処置を行うかどうかなどを勘案すべきです．しかし，かつて喧伝されたように，「DIC に対する補充療法は火に油を注ぐごとくである」という意見は全く当てはまりません．

血小板輸注は血小板減少を伴う DIC 患者に有効であり，特に出血症状を呈する場合には有効ですが，すでに出血症状を呈している患者では輸注効果は減弱します．血小板輸注のトリガーは患者の身体状態によりますが，一般的には，出血もしくは出血のリスクが高い患者で5万以下と考えられます[16] が，化学療法後の患者における RCT の結果[17] などをふまえると，出血のない患者では，もう少し低いトリガー値（1〜2万）が考慮されるでしょう．一方，PT や APTT の測定は補正効果のモニタリングに有効であり，フィブリノゲンの欠乏症は，フィブリノゲン製剤やクリオプレチピテートによって効果的に補正可能です．

▌凝固因子の補充

DIC の治療戦略の第一は基礎疾患の治療であることは言うまでもありませんが，基礎疾患の治療には時として困難さが伴い，DIC に伴う hypercoagulable state の改善が補助療法として必要です．基礎疾患の複雑多様さもあって，DIC の治療レジメンとしての補充療法に対しては，十分な臨床試験が行われて，それに基づくデータがあるわけでもないので，より一層の困難を伴います．

観血処置の場合には，予防的な FFP 輸注，血小板輸注が考慮されます．DIC 患者では，凝固因子・抗凝固因子・抗線溶因子の消費が非常に早いターンオーバーで起こっており，このような病態では「すべて」を含む FFP の輸注は血小板輸注，クリオプレチピテートなど，ともに一応理にかなっています．一方，出血傾向の乏しい慢性 DIC に対しては，検査データを正常化させるために漫然と投与すべきではありません．

▌抗凝固システムの再構築について教えてください

▌アンチトロンビン（AT）

ナチュラルな抗凝固因子を投与することによりバランスを改善することも，治療戦略の一つです．AT III は，先述したように強力な生理的凝固インヒビターであり，AT の DIC 患者への投与は検討が進んでいます．DIC 患者に対する検討は，主として基礎疾患として重症敗血症を有する患者に対してこれまで行われています．Kessler らは *Staphylococcus aureus* による DIC 動物モデルにおいて AT を 125〜1,000 IU/kg と大量に投与することにより，100％救命できることを明らかにしました[18]．Eisele らは，敗血症患者における AT 活性は 45.7±14.4％と低く，3,000 IU/day 4日間の大量投与で正常値（＞70％）まで上昇させうると報告しました[19]．

一方，近年行われた重症敗血症に対する多施設 RCT（KyberSept trial）では，AT の大量投与にもかかわらず，生存率の改善は得られませんでした[11]．ただし，症例を DIC 合併例に限定してサブ解析を行うと死亡率の改善が得られたことから[20]，DIC に対しては，やはり AT 補充による一定の効果があると考えられます．興味深いことに，ヘパリンを併用した群では有意に死亡率が上昇したことであり，併用に伴う出血有害事象の増加が懸念されます．

■ 活性化プロテイン C（APC）

　1987 年，濃縮 APC 製剤による DIC 動物モデルにおける著明な効果が報告されて以来[21]，DIC 症例に対する APC の効果が検討されてきました．わが国においても，血漿由来 APC 製剤の効果が，厚生省 DIC 診断基準で DIC と診断された症例に対して二重盲検 RCT によって検討されています（対照薬：未分画ヘパリン）．可溶性 fibrin monomer，D ダイマー，TAT，PIC は APC 群で有意に改善したものの，死亡率の改善にはいたりませんでした[22]．

　一方，重症敗血症症例を対象とした海外の多施設 RCT である PROWESS トライアルでは，有意に死亡率を改善することが示されています．この試験では，先に紹介した日本の試験の 10 倍の濃度の APC が使用されており，事実 APC 群では重症出血の合併が増加していました[23]．興味深いことは，当初観察された死亡率改善効果がフォローアップ期間を延長させるとみられなくなることです．その後実施された one-arm の ENHANCE トライアルでは，敗血症に対して早期に APC を投与しましたが，出血有害事象が増加しています[24]．最新の ADDRESS トライアル（APACHE II score＜25 の比較的重症度の軽い敗血症 2,640 例のプラセボ対照 RCT）においても，28 日後の死亡率の改善は有意にみられましたが，やはり重症出血の増加をみています[25]．

■ リコンビナントトロンボモジュリン（Recombinant thrombomodulin：rTM）

　TM は，本来，内皮細胞上のトロンビンレセプターの一つですが，ほかのトロンビンレセプターと異なる独特の機能をもちます[26]．ヒト TM の細胞外ドメインを遺伝子組み換え技術により発現・産生させた医薬品が rTM であり，液相においてもトロンビンに結合し，トロンビンの酵素特異性を変換，PC を APC に活性化させます（図 2）．In vivo での APC の半減期は非常に短いのに対して rTM の半減期は長く（t 1/2α 4 時間，t 1/2β 20 時間）[27]，非常にすみやかに可逆的なトロンビンとの複合体を形成し，かつリサイクルされることが知られています[28]．

　わが国で行われた rTM の多施設 RCT（対照薬：未分画ヘパリン）[12]では，厚生省 DIC 診断基準で診断された造血器悪性腫瘍もしくは感染症を基礎

疾患とする 234 例に対し検討が行われました．DIC スコアの改善を指標
とした DIC 離脱率はコントロール群に比して有意に高かったですが，死
亡率に関しては改善がみられたものの，統計学的な有意差をみるまでにい
たっていません．より顕著であったのは出血症状の消失率で，32.6%（rTM）
vs 13.3%（ヘパリン）と，造血器悪性腫瘍を基礎疾患とした DIC 症例で
有意でした（感染症では有意差はなし）．また，出血有害事象の発生率は
有意に低かったです．近年，多数例で実施された市販後調査の結果[29,30]
により，DIC における有用性が徐々に明らかになりつつあります．

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止血戦略におけるフィブリノゲン製剤の役割

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Summary

大量出血症例では，初期から凝固障害が存在する可能性が高いにもかかわらず，救命や循環動態改善を優先し，まず，赤血球濃厚液輸血や晶質液，人工膠質液の大量投与がおこなわれるために，ますます希釈性，消費性凝固障害を増悪させている可能性がある．止血のためにフィブリノゲン製剤（クリオプレシピテートやフィブリノゲン濃縮製剤）を用いると輸血量が大幅に減少するという報告があいついでいる．今後わが国においてフィブリノゲン製剤が導入されれば，大量出血においてはまずフィブリノゲン製剤を投与という日がくる可能性もある．

Key Words ●●●

・大量出血　・フィブリノゲン製剤　・止血　・フィブリノゲン

はじめに

大量出血症例では，初期から凝固障害が存在する可能性が高いにもかかわらず，救命や循環動態改善を優先し，まず，赤血球濃厚液輸血や晶質液，人工膠質液の大量投与がおこなわれるために，ますます希釈性，消費性凝固障害を増悪させている可能性がある．また出血性ショックやそれに伴う低体温，アシドーシスなどが，さらに凝固障害を増悪させている[1]．フィブリノゲンは，凝固因子のなかでの最終基質であり，血液凝固のメカニズムの最終段階でフィブリン（不溶性の網状線維素）となり，血栓を形成することで，止血に大変重要な役割を果たしている．したがって，ほかの凝固因子が活性化されても，フィブリノゲンがなければ，止血機構が十分にはたらかないこととなる．また，大量出血時に，凝固因子のなかで一番早期に止血に必要な血中濃度（約100mg/dL）を保てなくなるという特性をもち[2]．出血による急性低フィブリノゲン血症にいかに対応するかが，大量出血を早期に止血する際の大変重要な課題であることが指摘されている．このため，米国の輸血ガイドライン，輸血アルゴリズムについて記載されている文献では，心臓血管外科手術の大量出血などで低フィブリノゲン血症をきたした場合，止血のためにフィブリノゲン製剤（クリオプレシピテートやフィブリノゲン濃縮製剤）の使用が推奨されている[3][4]が，わが国では一般化されていない．しかし，その必要性は長きにわたり叫ばれつづけている．

本稿では大量出血においてのフィブリノゲン製剤の効能をエビデンスにもとづいて解説し，大量出血におけるフィブリノゲン製剤を用いた戦略にも言及する．
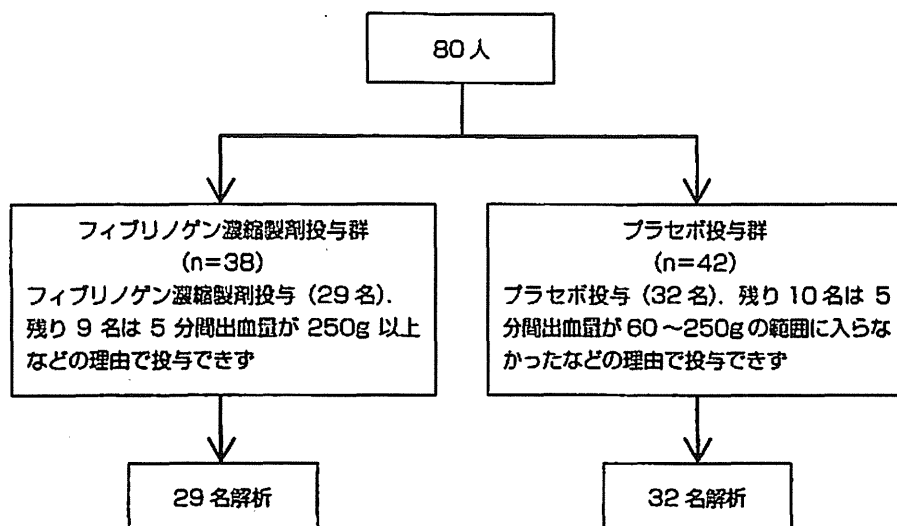
**図❶** Hannover 大学における二重盲検ランダム化比較試験

（Rahe-Meyer N *et al*, 2013[5]）より引用）

●1. 大量出血への対応の鍵は フィブリノゲン補充にあり

2013 年にドイツの Hannover 大学から衝撃的な論文が発表された[5]。この試験は一施設による 18 歳以上の人工心肺を用いた, 胸部および胸腹部大動脈置換術を受けた患者に対し, 人工心肺離脱後の 5 分間出血量が 60 ～250g であった場合にフィブリノゲン濃縮製剤もしくは生理食塩水を投与するという二重盲検ランダム化比較試験である（図❶）。フィブリノゲン濃縮製剤投与群では平均 8g のフィブリノゲンが投与されていた。驚くべきことに, フィブリノゲン濃縮製剤で介入した群では, 同種血輸血が回避された割合は 45％にも及ぶ一方で, プラセボ群において同種血輸血回避率は 0％であった。さらに, 術後 24 時間の総輸血量はプラセボ群と比較してフィブリノゲン濃縮製剤投与群で 85％削減が認められた。この研究においては, 血栓症や有害事象が増加していないかの検討もされているが, とくに有意な有害事象の増加はなかったとされる。

フィブリノゲン濃縮製剤については, 後ろ向き研究ではあるものの, 外傷の領域でも輸血頻度や患者予後改善につながるという報告もあり[6], この領域においてもフィブリノゲン製剤に対する今後の動向が注目される。

2014 年 9 月現在, わが国においては安全なフィブリノゲン濃縮製剤の供給が十分でなく, またクリオプレシピテートが供給されないことから, 大量出血時にはフィブリノゲン上昇を目指して新鮮凍結血漿（fresh frozen plasma：FFP）が投与されることが多い。当院においては輸血部でクリオプレシピテートを作製しており, とくに大血管外科において積極的に使用している。図❷ A に示すように, FFP480 を 4℃ で 30 時間程度かけて低温融解し, 遠心分離するとフィブリノゲンが多く含まれる白い沈殿物を認めるが, これがクリオプレシピテートである。つづいて, おもにアルブミンなどが含まれる上清部分を無菌操作で別バックに移動させ（図❷ B）, 残った 20～50mL をフィブリノゲンが濃縮されたクリオプレシピテートとして出庫している。原料の FFP に含まれるフィブリノゲンに個人差があるため, フィブリノゲン濃縮製剤と違いばらつきが多少あるものの, 3 パック（FFP480×3 本分）でおおむねフィブリノゲンは約 2g 程度含まれることになる。しかも容量としては 3 パックでせいぜい 150mL 程度なので, 容量負荷なくすぐに投与できるので, 大量出血時には重宝している。

今後, わが国においてフィブリノゲン製剤が導入されれば, 輸血のストラテジーが大きく変わる可能性がある。
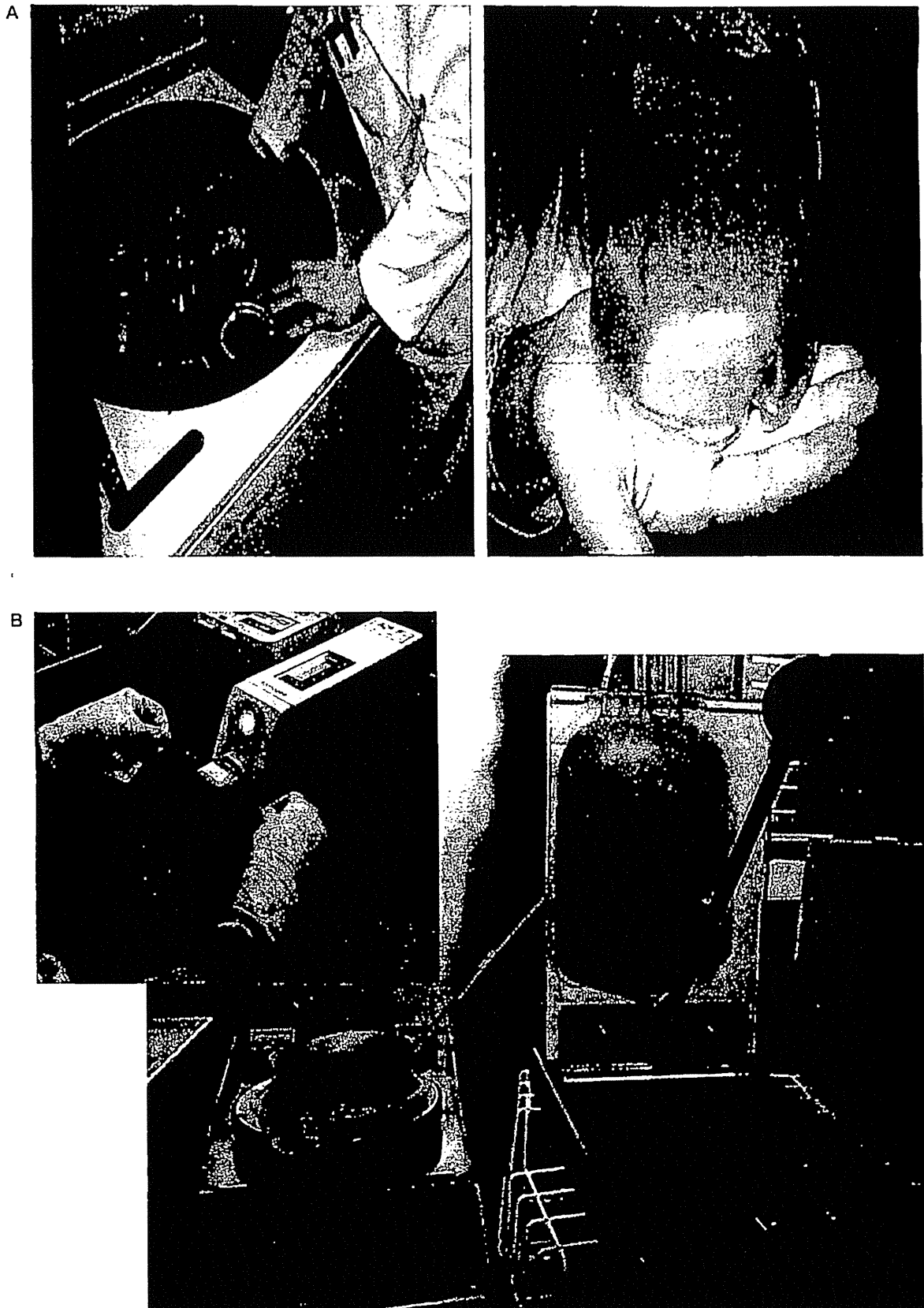
図❷　当院におけるクリオプレシピテート作製の様子

●2. 止血のメカニズム
〜なぜフィブリノゲン高値が 止血につながるか〜

フィブリノゲン値が高いほうが止血によいのは感覚的には理解できるが，ここはおさらいの意味もふくめて，止血のメカニズムからその理由を紐解いていく．止血のメカニズムは一次止血と二次止血に区別できる．一次止血においては，外傷などで血管内皮細胞が破綻し，露出したコラーゲンに von Willebrand factor（vWF）が結合する．血小板は血小板膜糖蛋白の GPIb（glycoprotein Ib）受容体を介して vWF と結合し，血管内皮細胞下組織に粘着，凝集して血小板血栓ができる．粘着した血小板は形態を変え，従来の円盤状の形態から偽足を出した形態となる．活性化した血小板はさまざまな顆粒を放出，血小板表面には血小板膜糖蛋白の GPIIb/IIIa 受容体が発現される．この GPIIb/IIIa に対してフィブリノゲンが結合し，血小板血栓を強固にする．つまり，フィブリノゲンは二次止血だけでなく，一次止血にも重要なはたらきを担っているのである．このようにフィブリノゲンは血小板血栓の安定化につながっているので，高いフィブリノゲン値は，血小板数の低下を補正できる可能性も指摘されている[7]．

二次止血の舞台は，凝集した血小板のリン脂質に富んだ膜である．内因系にせよ，外因系にせよ，凝固カスケードにはこのリン脂質と凝固因子が関与し，最終的にはトロンビンが産生されて，フィブリノゲンがフィブリンになって血栓が完成する．このように，一次止血においても血小板血栓を強固にする意味と，二次血栓において最終基質として血栓を完成させるという二つの側面から，止血においてフィブリノゲン値が高いことは重要であることが分かる．

●3. フィブリノゲン補充の カットオフ値は？

フィブリノゲン補充の目安として，多くのガイドラインで 100mg/dL があげられていることが多いが，じ
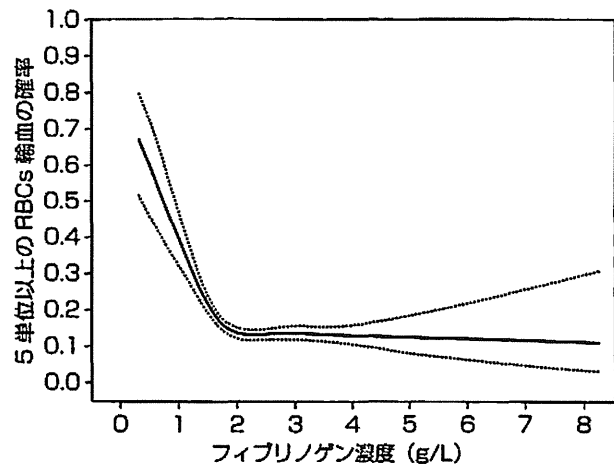
図❸ フィブリノゲン濃度と輸血量の関係
RBCs：red blood cells（赤血球）
(Karkouti K *et al.* 2013[8] より引用)

つはこの推奨についてのエビデンスレベルは低く，エキスパートオピニオンにもとづいている．しかしながら，このカットオフ値はじつは低すぎるのではないかという可能性が指摘されている．2013 年に出た報告[8]ではフィブリノゲン濃度 200mg/dL 未満群では大量輸血症例が増加していたとされる．（図❸）これは単施設後ろ向き観察研究で，人工心肺使用心臓手術 4,606 例を対象としている．フィブリノゲン濃度 200mg/dL 未満群は 42％の症例で，そのうち 18.9％が大量輸血を受けていた．一方，200mg/dL 以上群では 13.5％が大量輸血を受けていた（p＜0.0001）．リスク因子を調整後でも，大量出血のオッズ比は，1.8（1.4〜2.2）であり，傾向スコアを用いた解析でも 1.5（1.2〜2.0）であったとされる．

このほかにも，ドイツの Hannover 大学からの報告で，上行大動脈および大動脈弁置換術の患者を対象に，フィブリノゲン濃縮製剤を治療の最初に介入し，その後は輸血アルゴリズム（図❹）[9]に従うことで，総輸血量や術後出血量の大幅な減少が認められたとしているが，この試験においてはフィブリノゲン値が平均 200mg/dL 程の所でフィブリノゲン濃縮製剤が投与されている[9]．また，冠動脈バイパス術において出血を防ぐために必要なフィブリノゲン値は 100mg/dL よりずっと高いのではないかとする報告も散見されている[10]．麻酔科医の感