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Study protocol for assessment of the coagulation potential of concomitantly used factor VIII concentrates in patients with haemophilia A with emicizumab prophylaxis (CAGUYAMA Study): a multicentre open-label non-randomised clinical trial

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ABSTRACT

Introduction Emicizumab prophylaxis substantially reduces bleeding episodes in patients with haemophilia A (HA). The haemostatic efficacy of emicizumab in patients with HA is estimated as approximately 15% based on mimic activity of factor (F) VIII. Although it has been proven effective in preventing bleeding, its haemostatic effect during breakthrough bleeding or surgery is considered insufficient. Therefore, haemostatic management of emicizumab-treated patients with HA without inhibitors frequently requires FVIII replacement therapy. In haemostatic management of emicizumab-treated patients with HA, conventional FVIII dosage calculations are used in clinical practice without considering the coagulant effects of emicizumab.

Methods and analysis In the CAGUYAMA study, 100 patients with HA without inhibitors will be enrolled for a maximum duration of 1 year, and samples of 30 events following the concomitant use of FVIII concentrates (30±SU/kg) with emicizumab will be collected. An ‘event’ is defined as obtaining blood samples at preadministration and postadministration of FVIII concentrates during a breakthrough bleeding or a surgical procedure. Global coagulation assays will be used to measure the coagulation potential of the obtained samples. Clot waveform analysis (CWA) is used to identify the primary endpoint, that is, the degree of improvement in the maximum coagulation rate at preadministration and postadministration of fixed-dose FVIII concentrations. The parameter obtained from CWA, which is triggered by an optimally diluted mixture of prothrombin time reagent and activated partial thromboplastin time reagent, is reported to be an excellent marker for assessing the degree of improvement of the coagulation potential in emicizumab-treated plasmas.

Ethics and dissemination The CAGUYAMA study was approved by the Japan-Certified Review Board of Nara Medical University (Approval ID; nara0031). The study results will be communicated through publication in international scientific journals and presentations at (inter) national conferences.

Trial registration number jRCTs051210137.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The CAGUYAMA study is a multicentre, prospective trial including patients from 14 haemophilia treatment centres in Japan.
⇒ Recommended global coagulation assays are used to assess the coagulation potentials of the administration of FVIII concentrates concomitant with emicizumab prophylaxis in patients with haemophilia A without inhibitors.
⇒ The CAGUYAMA study is a single-arm trial; however, the effects of improvement in coagulation potential for patients with haemophilia A may be heterogeneous.

INTRODUCTION

Haemophilia A (HA) is a congenital bleeding disorder caused by a genetic abnormality of blood coagulation factor (F) VIII. The quality of life of patients with HA has been dramatically improved by the administration of FVIII concentrates at the time of bleeding or on a regular prophylaxis.12 Conversely, the half-life of coagulation factors in the blood has been lengthened with the extended half-life technology; however, they still require intravenous administration frequently. Another major challenge is the presence of anti-FVIII allo- geneic antibodies (inhibitors) in 20%–30% of patients with severe HA following replacement therapy.34
To overcome these unmet needs, a new concept of non-clotting factor emicizumab was developed. It has a long-lasting action (half-life of approximately 30 days) when administered subcutaneously to patients with HA in the presence or absence of FVIII inhibitors. Clinical studies have shown significant bleeding suppression, and emicizumab is currently covered in Japan by insurance for use as a regularly prophylactic bleeding control agent for patients with congenital HA. Although emicizumab has been proven effective in preventing bleeding, its haemostatic effect during breakthrough bleeding and surgery is sometimes considered insufficient. A previous study (UNEBI: Assessment of global coagulation function under treatment with 1011 emicizumab concomitantly with bypassing agents in haemophilia A with inhibitor) has already been conducted to evaluate the coagulation function of the combination of a bypass haemostatic agent during breakthrough bleeding and surgery in emicizumab-treated patients with HA with inhibitors. Conversely, there have been no studies evaluating the coagulation function of FVIII concomitant with emicizumab in patients with HA without inhibitors during haemorrhage and surgery. Haemostatic management of emicizumab-treated patients with HA often requires FVIII replacement therapy, and the haemostatic treatment is currently performed in clinical practice using conventional FVIII dosage calculations without considering the coagulation effect of emicizumab, which mimics approximately 15% of FVIII activity.

Therefore, this study aimed to evaluate the optimal dosage of FVIII (30±5 U/kg) for patients receiving emicizumab during breakthrough bleeding or during surgery. If haemostatic potential of emicizumab and FVIII exhibits additively, the FVIII dose could be reduced by approximately 10%–20%, and the dose of FVIII concentrates could be expected to decrease, which would be beneficial from medical and economic perspectives. Furthermore, since high FVIII activity level is reported to increase the risk of thrombosis, the use of the optimal FVIII dose (30±5 U/kg) during emicizumab treatment can be expected to reduce the risk of thrombosis. Therefore, we have started a clinical study entitled ‘Assessment of the coagulation potential of concomitantly used factor VIII concentrates in patients with haemophilia A with emicizumab prophylaxis’ (CAGUYAMA study), named after a famous historical mountain, Mt Kaguyama, located in the old Japanese capital (Kashiwara, Nara, Japan). This multicentre prospective trial, including patients from 14 major haemophilia treatment centres in Japan, would contribute to better understanding of the comprehensive haemostatic function of emicizumab and FVIII concentrates used concomitantly and might offer an optimal FVIII dosage for patients with HA receiving emicizumab.

It has been difficult to monitor the effects of emicizumab, and therefore, the global coagulation assays including thrombin generation assay, clot waveform analysis (CWA) and thromboelastometry were used for assessing the haemostatic potential of emicizumab in this study. Furthermore, a flow chamber-based system (total thrombus formation analysis system, TTAS, Fujimori-Kogyo, Tokyo, Japan) was also used to evaluate the global haemostasis under blood flow shear.

METHODS AND ANALYSIS

A summary of the study design is shown in Figure 1. This study has started on 20 December, 2021 and will end on 30 September 2025.

Aim

The CAGUYAMA study primarily aims to evaluate the global coagulation function under treatment with emicizumab concomitantly with FVIII concentrates in patients with HA without inhibitor and secondarily aims to determine an optimal FVIII dosage for patients receiving emicizumab during breakthrough bleeding and surgery.

Study patients

A total of 100 patients with HA without inhibitors will be enrolled in this study for a maximum duration of 1 year (from 17 January 2022 to 20 December 2022, and 30 events following the concomitant use of FVIII concentrates will be collected for a maximum duration of 3 years (from 17 January 2022 to 20 December 2024). Blood samples will be drawn at enrollment to assess the coagulation function during non-bleeding and stable condition. An ‘event’ means that both blood samples before and after infusion of FVIII concentrates are collected when haemostatic management is performed at breakthrough bleeding or surgery.

Inclusion criteria

1. Congenital HA without inhibitor* aged ≥ 4 years, considering the amount of blood drawn.

Figure 1 Schema of the study design. A total of 100 patients with haemophilia A treated with emicizumab but not with inhibitors will be registered. Patients should have blood samples withdrawn at enrollment to evaluate the single drug effect of emicizumab on global coagulation and should visit the participating institutions every 12 months. If breakthrough bleeding or surgical procedures occur, the physician decides to administer FVIII concentrates and blood will be withdrawn at two time points: before and 30 min after the first infusion. This study aims to obtain 30 events within 3 years.
*Without inhibitor denotes that patient’s medical record is negative for inhibitors at the start of or before emicizumab treatment. The criteria for negative inhibitor status (<0.6 BU/mL) are in accordance with each institution’s criteria.

2. Patients treated with emicizumab (≥5 times) based on the latest package insert.

3. Patients who were informed about the content of this clinical study and provided written consent (consent form in online supplemental material).

4. Patients who can comply with the planned procedure in this clinical study.

**Exclusion criteria**

1. Patients with difficulty making regular visits and/or visits at the time of an event.

2. Patients with other diseases characterised by abnormal liver injury; aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) >5 fold of the upper limit of the normal range) or low platelet counts (<100 ×10⁹/L).

3. Patients with difficulty undergoing blood collection.

4. Patients judged by the investigator to be inappropriate to enter this study for some other reasons (ie, patients who were not able to understand the content of this clinical study).

**Emicizumab prophylaxis**

Patients must comply with the last updated recommended use of emicizumab prophylaxis. As of October 2019, the recommended dose is 3 mg/kg by subcutaneous injection once weekly for the first 4 weeks, followed by one of three options as mentioned below:

- 1.5 mg/kg, once weekly.
- 3.0 mg/kg, once every 2 weeks
- 6.0 mg/kg, once every 4 weeks.

**Assessment schedule**

**Regular visit and blood sample collection**

Blood samples will be drawn at enrolment to assess the coagulation function during patient with non-bleeding and stable status. Patients visit the participating institutions (14 hospitals in Japan) every 12 months to ensure that their condition is stable and that emicizumab is administered as scheduled.

**Therapeutic intervention in breakthrough bleeding**

This study does not have a strict protocol for every concomitant use of FVIII concentrates; however, when an event occurs, that is, collection of blood samples before and after an infusion of FVIII concentrates, a strict dosing protocol is mandatory.

When the first infusion of FVIII concentrates is insufficient for haemostasis, further treatment should be considered. Assessment of treatment of acute joint/muscle bleeds was followed by the definition of ISTH (International Society on Thrombosis and Haemostasis).

Subsequent FVIII concentrates infusions should comply with the treatment guidance published by a vendor in Japan.

**Therapeutic intervention in perioperative management**

This study does not have a strict protocol for each concomitant use of FVIII concentrates. When an event occurs, that is, collection of blood samples before and after an infusion of FVIII concentrates, a strict dosing protocol is mandatory.

When patients are scheduled for an operation and the investigator decides that FVIII concentrates are needed for perioperative haemostatic management, FVIII concentrates must be chosen, and the first dose must be strictly set at 30±5 U/kg. However, if the physician determines that the operation requires the patient to receive >30 ± 5 U/kg of FVIII concentrates, the event will not be registered.

When the first infusion of FVIII concentrates is not sufficient for perioperative management, further treatment should be considered. Assessment of haemostatic response for surgical procedures was followed by the definition of ISTH.

Subsequent FVIII infusions should comply with the treatment guidance published by a vendor in Japan.

**Blood sample collection in breakthrough bleeding or perioperative management**

Blood samples are taken by the investigator at two time points: (1) before the first infusion of FVIII concentrates (usually when the venous access route is secured) and (2) 30±15 min after the first infusion of FVIII concentrate (do not use the same venous access route of FVIII concentrate infusion).

This study aims to obtain 30 events (30 sample pairs of two time point blood samples before and after FVIII infusion) from 100 registered patients within 3 years.

**Sample handling procedure and sample volume**

Blood samples at enrolment or events (see the Blood sample collection section) are preserved as it is for complete blood counts (CBCs) or centrifuged (collect supernatant) for plasma examinations. Both are sent to a laboratory service company (LSI Medience, Japan), and CBC and standard plasma tests were immediately performed for coagulation and fibrinolysis (see the Standard laboratory tests section). A part of plasma samples was stored in a deep freezer at −80°C and transferred to Nara Medical University for global assays (see the
Global assays section). The total sample volume in each venepuncture is 15 mL. Additionally, only event samples from Nara Medical University of ≥6 mL are used to perform additional global assays using whole blood (see the Whole blood global assays section). Therefore, the total sample amount at an event in Nara Medical University is 21 mL for each venepuncture.

Concomitant therapy and post-treatment
Drugs listed as contraindications for the combined use in package inserts of the protocol drugs are prohibited. No concomitant therapy of coagulation-associated drugs (ie, tranexamic acid) is permitted for a 30 min duration of the event (between the two time points of blood collection). Whether the patient should receive additional treatment depends on the investigator’s preference. When the patient has already been infused at home with FVIII concentrates, the first infusion of FVIII concentrates at the hospital can be deemed as an event if the interval is at least 4–5 times the half-life of FVIII concentrates.

Observation and test points
At the beginning of the registry, background information of patients, including age, HA severity, history and drug use history of FVIII concentrates, is recorded in the electronic data capture (EDC) system, and blood samples are obtained for blood analysis information in stable condition while participating in this study. The medical history associated with bleeding is recorded at regular visits every 12 months.

When the event, breakthrough bleeding or perioperative management occurs, two-point blood samples before/after the first infusion of FVIII concentrates is obtained. Blood examinations (see the Examination lists section) of this study are performed later to evaluate the efficacy of therapy.

Detailed records of the whole treatment course associated with events (breakthrough bleeding or perioperative management) must be obtained, including the infusion product name and date and dose of all FVIII concentrates including those used at home.

Investigators should evaluate the efficacy and adverse events for both the protocol (a single infusion of FVIII concentrates) and whole treatment course through routine clinical workup, including physical examination and clinical laboratory tests.

Examination lists

Standard laboratory tests
The white blood cell, red blood cell, haemoglobin, haematocrit, platelets, AST, ALT, lactate dehydrogenase, total bilirubin, indirect bilirubin, blood urea nitrogen, creatinine, fibrinogen, fibrinogen digestive products (FDPs) and D-dimer levels were measured.

Global assays
CWA and TGT (thrombin generation test) are performed in the presence of anti-Emi-mAb.

Whole blood global assays
Rotational thromboelastometry (ROTEM), T-TAS and whole blood platelet aggregometry (Multiplate) are performed with the event samples only at the Nara Medical University. ROTEM is performed in the presence or absence of anti-Emi-mAb.

Outcome measures
To date, no standard assay has been established to evaluate emicizumab and FVIII concentrates. Recently, CWA, TGT and ROTEM have been reported useful for evaluating the global coagulation efficacy of emicizumab.

Figure 2  Scheme of end-point parameters. The primary end-point is the degree of improvement in the maximum coagulation rate by clot waveform analysis at preadministration and postadministration of FVIII concentrates. Furthermore, we attempted to visualise the relative contribution of emicizumab and FVIII concentrates to the magnitude of coagulation function using anti-Emi-mAb. anti-Emi-mAb, anti-emicizumab monoclonal antibodies.
performed with some commercial, fully automated coagulation machines. Therefore, we expect that CWA is the most versatile assay. Furthermore, we attempted to visualise the magnitude of how emicizumab and FVIII concentrates distinctly contribute to the comprehensive coagulation function using anti-Emi-mAb (figure 2).

Primary end-point
The primary end-point is the degree of improvement in the maximum coagulation rate by CWA at predadministration and postadministration of fixed-dose FVIII concentrates. A parameter of CWA, ad|m1|, that is, the adjusted maximum velocity, triggered by optimally diluted prothrombin time/aPTT (activated partial thromboplastin time) reagents, is reported to be an excellent marker to understand how the coagulation function is improved in emicizumab-treated plasma (figure 2).

Secondary end-points are as follows
2. Comparative evaluation with and without adding anti-Emi-mAb for changes in comprehensive coagulation parameters at predadministration and postadministration of FVIII concentrates.
3. Efficacy of emicizumab with and without adding anti-Emi-mAb using comprehensive coagulation tests in the samples at enrolment.
4. Evaluation of general laboratory tests at predadministration and postadministration of FVIII concentrates and at enrolment.
5. Efficacy of emicizumab with the ex vivo addition of FVIII concentrates using comprehensive coagulation tests in the samples at enrolment.

Exploratory end-point
The exploratory end-point is the relationship between the plasma concentration of emicizumab and comprehensive coagulation potential in the samples at enrolment.

Safety assessment
The clinical haemostatic efficacy of concomitantly using emicizumab and FVIII concentrates will be evaluated. Additionally, laboratory data, including the platelet count, FDP and D-dimer, will be examined during the whole treatment course.

Severe adverse event
Severe adverse event (SAE) will be immediately reported to the corresponding investigator, regardless of the possibility of a causal link to emicizumab or FVIII concentrates. It will also be announced to Nara Medical University Certified Review Board and Chugai Pharmaceutical Co. A meeting of the Efficacy and Safety Assessment Committee will be held as needed.

Adverse event of special interest
Adverse event of special interest (AESI) refers to an adverse event that has been strongly associated with emicizumab based on previous clinical trials. The CAGUYAMA study defines three AESI: (1) systemic hypersensitivity or anaphylactic reaction, (2) thromboembolism and (3) TMA (thrombotic microangiopathy) including thrombotic thrombocytopenic purpura or haemolytic uremic syndrome. When AESI occurs, the reporting procedure will be the same as for SAE.

Statistical analysis
Sample size validity
The primary end-point of this study is the increase in maximum coagulation velocity in CWA at predadministration and postadministration of FVIII concentrates during regular emicizumab administration. A previous study18 that measured the maximum coagulation velocity (ad|m1|) after FVIII administration under emicizumab prophylaxis revealed the SD of ad|m1| did not significantly change with FVIII dose, ranging from 0.6 to 0.7. In this study, when estimating the mean ad|m1| value after FVIII administration with a 95% CI of±0.25 (range of 0.5), assuming an SD of 0.7, the target estimation accuracy would be achieved with samples from 30 events.

Currently, 46 patients with HA without inhibitor on regular emicizumab prophylaxis were followed at Nara Medical University; among them, 5 had a breakthrough bleeding and 6 had surgery in 2020 (11/46; 24%). Assuming that patients can be observed for approximately 2 years, if 100 patients can be enrolled, 30 of them will be expected to have a bleeding event eligible for this study. If 30 patients can be collected without a 3-year observation period, the observation period will end when the data collection required for the primary evaluation has been achieved.

Statistical analysis plan
For the primary end-point, the degree of parameter improvement before and after administration of fixed-dose FVIII concentrates will be estimated after the administration level with 95% CI.

For the first secondary end-point, the physician will record the clinical evaluation of haemostasis status. For the second secondary end-point, each 95% CI with and without adding anti-Emi-mAb for changes in comprehensive coagulation parameters before and after administration of FVIII concentrates will be estimated. For the third secondary end-point, global assay parameters will be obtained with and without adding anti-Emi-mAb in the samples at enrolment, and each 95% CI will be estimated. For the fourth secondary end-point, general laboratory tests should be performed before and after administration of FVIII concentrates and at enrolment. Furthermore, for the fifth secondary end-point, global assay parameters will be obtained with the ex vivo addition of FVIII concentrates in the samples at enrolment.

For the exploratory end-point, the relationship between global assay parameters and plasma concentrations of emicizumab will be obtained in the samples at enrolment, and each 95% CI will be estimated.

We have recently reported that CWA, TGT and ROTEM were useful in monitoring the emicizumab and concomitant emicizumab and FVIII concentrate use in emicizumab-treated patients. 17 18 20 26 In this study, we determine the FVIII dosage to be used during breakthrough bleeding and surgery in patients with HA without inhibitor who are treated with emicizumab and monitor their coagulation potential before and after administration using these global coagulation assays to determine the relationship with clinical symptoms. We expect the CAGUYAMA study to obtain evidence for the safer use of FVIII with emicizumab in patients with HA without inhibitor.

The CAGUYAMA study has several limitations. The intervention is limited because the safety of registered patients is prioritised. Blood is collected only once in one bleeding or surgical episode. Global assays using whole blood are limited to one institution, whereas plasma assays such as TGT and CWA can be performed for all frozen samples. We do not compare the FVIII concentrates used (recombinant FVIII or plasma-derived FVIII and standard half-life FVIII or extended half-life FVIII).

DISCUSSION

The FVIII function-replacement bispecific antibody (emicizumab) is an innovative non-coagulant agent that markedly controls bleeding in patients with HA with regular subcutaneous administration. However, the coagulation efficacy of emicizumab alone is insufficient for haemostatic management of breakthrough bleeding and during surgery, and concomitant management with conventional FVIII concentrates is necessary. However, the optimal FVIII dose for haemostatic management with FVIII concentrates in patients without inhibitors treated with emicizumab has not been investigated, and the same FVIII dose has been administered when emicizumab is not administered. Therefore, the optimal FVIII dose should be considered in the context of the haemostatic coagulation ability of emicizumab when administered with emicizumab.
REFERENCES


CAGUYAMA Study
Creation Date: July 1, 2021 Version 1.0

Consent Form

Title of Study: "Assessment of the coagulation potential of concomitantly used factor VIII concentrates in patients with haemophilia A with emicizumab prophylaxis (CAGUYAMA Study): A multi-center open-label non-randomized clinical trial"

I have received and fully understood this written explanation of my participation in this study, and hereby give my consent to participate in this study of my own free will. As proof of my consent, I sign below and receive a copy of the explanation and consent form.

I agree to the secondary use of the specimens and test data obtained in this study within the range where personal information is protected.

□ I agree to the secondary use.  □ I do not consent to the secondary use.

Date of Consent:
Patient's Name:
Alternate Patient's Name:
*If the consent form or assent document is not signed by the patient

□ Willingness to participate verbally
□ No verbal willingness to participate

Explaination Date:
Name of physician in charge:

Note: If the date of consent and procedures related to this research are to be performed on the same day, please indicate the time (in 24-hour notation). If the consent form/explanation document has been revised, it is not necessary to indicate the time of the revision.