Regression Analysis to Estimate the Factor VIII Activity of Patients with Hemophilia A Without Inhibitor who Received Emicizumab Therapy

Yuki Hatayama, PhD¹,², Toru Motokura, MD, PhD²,³, Yuzuru Hosoda, MD³, Sayaka Suzuki, MD³, Hiroya Namba¹, Konami Kato¹, Nao Kojima¹, Takuya Horie, PhD¹, Takuya Iwamoto¹, Noriko Yamashita¹, Hitomi Ichikawa¹, and Tetsuya Fukuda, MD, PhD¹,³

Abstract

Background: Emicizumab, a bispecific monoclonal antibody for hemophilia A (HA), has strong pharmacodynamic effects in several coagulation assays resulting in dosing difficulties with Factor VIII (FVIII) concentrates during bleeding emergencies.

Materials and Methods: Single and multiple regression models were studied to estimate FVIII activity using 27 archived plasma samples from three patients with HA without inhibitor under emicizumab treatment. Explanatory variables were FVIII chromogenic assay (CSA), Adimin1, Adimin2, the number of seconds of APTT, and the FVIII one-stage assay (OSA), which were measured without idiotype antibodies. The response variable was FVIII OSA measured with idiotype antibodies.

Results: In the simple linear model, the FVIII CSA regression coefficient was 1.04 and the intercept was $-14.55$ ($r^2 = 0.95; p < 0.001$). In the multiple regression model, FVIII OSA and FVIII CSA were selected based on the Akaike Information Criterion, with regression coefficients of 1.74 and 1.15, respectively, and an intercept of $-92.03$ ($r^2 = 0.96, p < 0.001$).

Conclusions: The regression models can estimate the FVIII:C levels in patients with HA receiving emicizumab and would be useful in a bleeding emergency and/or surgery.

Keywords

emicizumab, factor VIII, chromogenic assay, one-stage assay, clot waveform analysis, regression analysis

Date received: 29 December 2021; revised: 29 January 2022; accepted: 7 February 2022.

Background

Hemophilia A (HA) is an X-linked hereditary bleeding disorder caused by deficiency of coagulation factor (F) VIII activity. Under physiological conditions, FVIII is activated by thrombin and subsequently acts as a cofactor for activated FIX (FIXa) to facilitate the activation of FX. Consequently, reduced FVIII activity leads to diminished generation of FXa, resulting in insufficient coagulation potential and bleeding complications.¹,² HA is classified into three types based on FVIII activity levels: mild HA (5 to < 40% FVIII activity); moderate HA (1 to < 5%); and severe HA (< 1%).³ Usually, HA is treated with intravenous administration of recombinant or plasma-derived FVIII concentrates to restore hemostasis.⁴ Routine supplementation with FVIII has two major drawbacks aside from its expense: the development of inhibitors and the need for frequent venous access for FVIII injection. Inhibitors precluding the use of FVIII make it difficult to control bleeding because alternative

¹ Division of Clinical Laboratory, Tottori University Hospital, Yonago, Tottori, Japan
² Division of Clinical Laboratory Medicine, Department of Multidisciplinary Internal Medicine, School of Medicine, Tottori University Faculty of Medicine, Yonago, Tottori, Japan
³ Department of Hematology, Tottori University Hospital, Yonago, Tottori, Japan

This study was presented at the 31st regional (virtual) congress of the International Society of Blood Transfusion (ISBT in Focus) on June 2-8, 2021. It was selected as one of the best poster presentations and selected for the main program (Vox Sanguinis. 2021;116[S1]:107-108; https://doi.org/10.1111/vox.13117).

Corresponding Author:
Yuki Hatayama, Division of Clinical Laboratory, Tottori University Hospital, 36-1 Nishi-cho, Yonago, Tottori 683-8504, Japan.
Email: y-hata@tottori-u.ac.jp
treatment agents (such as recombinant activated FVII and activated prothrombin complex concentrates) have shorter half-lives, cost more than FVIII, and are not always effective.5–8

A new treatment option for patients with HA with FVIII inhibitor is the subcutaneous, 1.5 mg/kg once-weekly administration of emicizumab, a novel, bispecific, humanized monoclonal antibody, which has been approved in several countries for routine prophylaxis in patients with HA with FVIII inhibitor.5 Emicizumab mimics the function of FVIIIa by bridging FIXa and FX to restore effective hemostasis.5 Although emicizumab and FVIII show some functional similarities, several key differences influence the results of standard laboratory assays when conducted in the presence of emicizumab. This can result in misleading interpretation of coagulation assays in patients treated with emicizumab.9 Emicizumab has strong pharmacodynamic effects on activated partial thromboplastin time (APTT)-based assays and chromogenic FVIII assays (CSA) using human FX/FIXa.10 As a result of the interference of emicizumab with APTT, the FVIII one-stage assay (OSA) reports FVIII activities exceeding 150% in the presence of emicizumab.11,12 Consequently, all variations of OSA and Bethesda assays using OSA to interpret results should be avoided in the management of patients receiving emicizumab. The FVIII CSA using bovine FX/FIXa can measure FVIII:C accurately because emicizumab does not interfere with bovine factors. However, the availability of bovine reagents is now limited in Japan,9 hence, laboratory tests are needed to estimate FVIII:C for patients with HA requiring FVIII infusions.

In a previous study, two anti-idiotype monoclonal antibodies (mAbs) were developed (rcAQ8, a mAb binding to the anti-FIXa arm of emicizumab; and rcAJ540, an anti-FX arm mAb) and assessed using APTT and FVIII OSA.13 The addition of both rcAQ8 and rcAJ540 to samples containing emicizumab almost completely eliminated its binding potential for human FIXa and FX. This implies that the assays measured FVIII activity as they would in the absence of emicizumab.13 However, these antibodies are not commercially available, and one needs to apply to the pharmaceutical company to obtain them. Therefore, FVIII activity cannot be accurately measured in the case of a bleeding emergency in patients with HA receiving emicizumab when FVIII concentrates are administered. In this study, we studied regression models to estimate FVIII activity in the presence of emicizumab to address this issue.

Materials and Methods

Patients and Clinical Samples

Twenty-seven archived plasma samples from three patients (age range, 43–78 years) with severe HA without inhibitor for FVIII at the Tottori University Hospital, Tottori, Japan, from 2019 to 2021 were used in this study. These patients were given standard FVIII concentrates because of orthopedic surgery, prostate biopsy, or appearance of bleeding events. All samples were collected during the steady state of emicizumab therapy and using standard venipuncture blood collection tubes containing one-tenth volume of sodium citrate (3.2%/0.109 M) to provide a final citrate concentration of 0.32%/0.0109 M. Platelet-poor plasma was obtained after centrifugation of citrated whole blood for 15 min at 1500 × g. All plasma samples were stored at −80 °C and thawed at 37 °C immediately prior to the assays. All samples were analyzed within 6 months. This study was approved by the Ethics Committee at Tottori University Faculty of Medicine (approval number: 19A056).

Coagulation Assays

Coagulation assays for APTT (Thrombocheck APTT-SLA using ellagic acid and silica as triggers; Sysmex, Kobe, Japan), FVIII OSA (FVIII-deficient plasma; SIEMENS, Marburg, Germany), and FVIII chromogenic assay (CSA) (Revohem FVIII chromogenic; Sysmex, Kobe, Japan) using human FX and FXa were performed using commercially available diagnostic kits according to instructions provided by the manufacturers. Additionally, we used the parameters Adjusted [min1] (Ad[min1]) and Adjusted [min2] (Ad[min2]), which are obtained from the APTT clotting wave form calculated by the blood coagulation analyzer CN-6000 (Sysmex, Kobe, Japan). With the modified clot waveform analysis (CWA), the minimum transmittance (0%) was set in the immediate post coagulation phase, and Ad[min1] and Ad[min2] were defined accordingly as the maximum coagulation velocity and acceleration, respectively, obtained from the first or second derivative of this adjusted clot waveform.14 Samples from patients with HA with emicizumab treatment were analyzed with or without anti-idiotype antibodies (both rcAQ8 and rcAJ540).13 Plasma and antibodies were mixed at a ratio of 20:1. These anti-idiotype antibodies were kindly provided by Chugai Pharmaceutical, Tokyo.

Statistical Analysis

The following values measured without antibodies were used as explanatory variables: FVIII CSA, Ad[min1], Ad[min2] and the number of seconds of APTT (APTT [s]) and FVIII OSA. FVIII OSA measured with antibodies was the response variable in the simple and multiple regression models. In the multiple regression (MR) analysis, variables were selected based on the Akaike Information Criterion (AIC) using the stepwise method in both directions, and the significance level of each variable was set to P < 0.05. Statistical analysis was performed using EZR software (ver. 1.54, Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a Japanese user interface for R (ver. 4.0.3, The R Foundation for Statistical Computing, Vienna, Austria).15

Results

To evaluate the linearity of FVIII:C measured with anti-idiotype antibodies, we formulated different concentrations of FVIII by mixing two plasma samples, before and after administration of FVIII concentrates. The R² between FVIII:C values measured in vitro with antibodies and expected FVIII:
C values was 0.997 (Figure 1), showing a good linearity over the clinical range. Since FVIII OSA exceeded the upper limit of measurement, the APTT (s) after mixing patient plasma and FVIII-deficient plasma was used in the regression analysis.

In the simple linear regression (SLR) model, Ad[\text{min}1], Ad[\text{min}2], FVIII OSA, and FVIII CSA were significant variables, with FVIII CSA being the most significant (regression coefficient = 1.04, intercept = −14.55; adjusted R² = 0.95; p < 0.001; AIC = 125.69). FVIII:C estimation by Ad[\text{min}1], Ad[\text{min}2] or FVIII OSA was accurate, giving negative values in case of FVIII under 20%. In the MR model, FVIII OSA and FVIII CSA were selected as explanatory variables based on AIC, using the stepwise method with both directions. The regression coefficient estimates of FVIII OSA and FVIII CSA were 1.74 and 1.15, respectively, and the intercept of this regression coefficient was 1.74 for MR, 0.96 for SLR, with FVIII CSA being the most significant variables, and FVIII OSA and FVIII CSA were significant variables, in the SLR model, which indicated the differences between FVIII and emicizumab. The absolute value of the intercept would be lower in patients which is estimated to be approximately 15%. Thus, the absolute value of the intercept would be lower in patients during the saturation phase of emicizumab treatment.

In the MR model, the addition of FVIII OSA improved the estimation slightly and the regression coefficient was 1.74 for APTT (s) in OSA. The coefficient means that FVIII:C lengthens APTT in OSA. Because FVIII has a higher affinity for FIXa/FX than for emicizumab, it seems that the action of emicizumab is reduced in the presence of both, and the reduction in APTT by emicizumab is reversed. Ad[\text{min}1] and Ad[\text{min}2] were also significant variables in the SLR model, which should reflect the differences between FVIII and emicizumab. Because emicizumab does not require an activation step by thrombin-mediated proteolysis to obtain its cofactor activity, the coagulation velocity and acceleration of emicizumab are slower than those of FVIII.

Figure 1: Evaluation of the linearity of FVIII:C measured by one-stage assay with neutralizing anti-idiotypic antibodies. Two plasma samples (which were taken before and after administration of FVIII concentrates) were mixed to prepare a serial dilution, and FVIII:C was measured by one-stage assay with (open circles) or without (closed circles) antibodies.

Discussion

Although emicizumab mimics the activity of FVIIIa, the two molecules have no structural homology and have several biochemical and functional differences. Emicizumab has a half-life of approximately 30 days and achieves stable mean plasma concentrations by week 4 of treatment in clinical studies. Conversely, FVIII has a half-life of approximately 12 h, which is dependent on several inter- and intra-individual factors.

Since the coagulation function during administration of emicizumab is estimated to be approximately 15% of FVIII equivalent activity, it is necessary to supplement FVIII concentrates at the time of bleeding depending on the severity. However, emicizumab influences the results of several standard laboratory assays and can result in misleading interpretation of coagulation assays in emicizumab-treated patients. In this study, we studied regression models to estimate FVIII activity in patients with HA (without inhibitor) who received emicizumab therapy. Regression analysis enables the identification and characterization of relationships among multiple factors. It also enables the identification of prognostically relevant risk factors and the calculation of risk scores for individual prognostication. In the SLR models, Ad[\text{min}1], Ad[\text{min}2], FVIII OSA, and FVIII CSA were selected as explanatory variables based on AIC, using the stepwise method with both directions. The regression coefficient estimates of FVIII OSA and FVIII CSA were 1.74 and 1.15, respectively, and the intercept of this model was −92.03 (Table 1). In case of SLR (Figure 2A–E), FVIII CSA showed the smallest AIC and this model estimated FVIII better than the other models. The addition of FVIII OSA to FVIII CSA improved the estimation slightly (Figure 2F). This model had the smallest AIC and fitted well (adjusted R² = 0.96; p < 0.001; AIC = 120.3). The variance inflation factors were 2.05 and 2.05, for MR, respectively.

Figure 1. Evaluation of the linearity of FVIII:C measured by one-stage assay with neutralizing anti-idiotypic antibodies. Two plasma samples (which were taken before and after administration of FVIII concentrates) were mixed to prepare a serial dilution, and FVIII:C was measured by one-stage assay with (open circles) or without (closed circles) antibodies.
Table 1. Simple and multiple regression analyses of plasma samples from patients with hemophilia A without inhibitor for FVIII.

|                  | Simple regression | Multiple regression |
|------------------|-------------------|---------------------|
|                  | PRC (95% CI)      | Intercept           | r²       | PRC (95% CI)      | Intercept           | r²       | AIC selection |
|                  |                   |                     |         |                   |                     |         |               |
| APTT             | −9.11 (−20.52-2.30) | 244.97              | 0.998   | 1.2 (−3.22-5.63)  | −246.76             | 0.96     | ns            |
| Ad[min1]         | 46.15 (32.19-60.12) | −369.81             | 0.64    | 21.36 (−46.23-88.97) | −57.99            | ns     |
| Ad[min2]         | 209.32 (139.65-279.0) | −235.79             | 0.60    | (−362.16-246.16)  | 2.24               | ns     |
| FVIII OSA        | −7.49 (−11.31-3.67) | 351.68              | 0.39    | (−0.20-4.69)      | 1.74 (0.44-3.06)   | 0.96    |
| FVIII CSA        | 1.04 (0.95-1.13)   | −14.55              | 0.95    | 1.05 (0.83-1.27)   | 1.15 (1.04-1.27)   |         |

APTT, activated partial thromboplastin time; OSA, one-stage assay; CSA, chromogenic assay; PRC, partial regression coefficient; CI, confidence interval; AIC, Akaike Information Criterion; ns, not selected.

Figure 2. (A–F) Estimation of FVIII:C by each regression model. The X-axis denotes FVIII:C measured by one-stage assay with antibodies, and the Y-axis denotes the FVIII:C estimated by each model. Estimated FVIII:C values were calculated with the partial regression coefficients and intercepts as shown in Table 1 by simple regression models (A–E) and a multiple regression model (F). In the multiple regression model, variables were selected based on the AIC using the stepwise method in both directions.

In patients with HA with inhibitor, bypassing agents such as recombinant FVIIa and activated prothrombin complex concentrate are used, especially with high levels of inhibitors where FVIII replacement is ineffective. Estimation of FVIII:C is then redundant. However, in patients with lower levels of inhibitor, the FVIII concentrates are still effective and preferable because of cost. The current model using FVIII OSA and FVIII CSA may be helpful but should be investigated further.

Conclusions
FVIII:C in patients with HA under emicizumab therapy can be estimated using a regression model with FVIII CSA. The
regression model would be useful in responding to a bleeding emergency and/or surgery when specific reagents are not available.

Acknowledgments
We would like to thank Editage (www.editage.com) for English language editing.

Authors’ Contributions
Y.H. designed and performed the study and wrote the first draft of the manuscript; Y.H. and S.S. treated and managed the patients; K.K. contributed essential reagents and tools; H.N., N.K., T.I., T.H., N.Y., and H.I. acquired and analyzed the data; T.M. and T.F. supervised the study and reviewed and edited the manuscript.

Research Ethics and Patient Consent
This study was approved by the Ethics Committee at Tottori University, Faculty of Medicine (approval number: 19A056). Informed consent was obtained through an opt-out approach.

Availability of Data and Materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD
Yuki Hatayama https://orcid.org/0000-0002-5256-1913

References
1. Tiede A, Abdul Karim F, Jiménez-Yuste V, et al. Factor VIII activity and bleeding risk during prophylaxis for severe hemophilia A: a population pharmacokinetic model. Haematologica. 2021;106(7):1902-1909.
2. Samuelson Bannow B, Recht M, Négrier C, et al. Factor VIII: long-established role in haemophilia A and emerging evidence beyond haemostasis. Blood Rev. 2019;35:43-50.
3. Srivastava A, Santagostino E, Dougall A, et al. WFH Guidelines for the management of hemophilia, 3rd edition. Haemophilia. 2020;26(6):1-158.
4. Franchini M, Mannucci PM. Hemophilia A in the third millennium. Blood Rev. 2013;27(4):179-184.
5. Kitazawa T, Igawa T, Sampei Z, et al. A bispecific antibody to factors IXa and X restores factor VIII hemostatic activity in a hemophilia A model. Nat Med. 2012;18(10):1570-1574.
6. Young G, Auerswald G, Jimenez-Yuste V, et al. When should prophylaxis therapy in inhibitor patients be considered? Haemophilia. 2011;17(5):e849-e857.
7. Astermark J, Donfield SM, DiMichele DM, et al. A randomized comparison of bypassing agents in hemophilia complicated by an inhibitor: the FEIBA NovoSeven comparative (FENOC) study. Blood. 2006;109(2):546-551.
8. Leissinger C, Gringeri A, Antmen B, et al. Anti-Inhibitor coagulant Complex prophylaxis in hemophilia with inhibitors. N Engl J Med. 2011;365(18):1684-1692.
9. Müller J, Pekrul I, Pützsch B, Berning B, Oldenburg J, Spannagl M. Laboratory monitoring in emicizumab-treated persons with hemophilia A. Thromb Haemostasis. 2019;119(9):1384-1393.
10. Adamkewicz JJ, Chen DC, Paz-Priel I. Effects and interferences of emicizumab, a humanised bispecific antibody mimicking activated factor VIII cofactor function, on coagulation assays. Thromb Haemostasis. 2019;119(7):1084-1093.
11. Al-Samkari H, Croteau SE. Shifting landscape of hemophilia therapy: implications for current clinical laboratory coagulation assays. Am J Hematol. 2018.
12. Uchida N, Sambe T, Yoneyama K, et al. A first-in-human phase 1 study of ACE910, a novel factor VIII-mimetic bispecific antibody, in healthy subjects. Blood. 2016;127(13):1633-1641.
13. Nogami K, Soeda T, Matsumoto T, Kawabe Y, Kitazawa T, Shima M. Routine measurements of factor VIII activity and inhibitor titer in the presence of emicizumab utilizing anti-idiotype monoclonal antibodies. J Thromb Haemost. 2018;16(7):1383-1390.
14. Nogami K, Matsumoto T, Tabuchi Y, et al. Modified clot waveform analysis to measure plasma coagulation potential in the presence of the anti-factor IXa/factor X bispecific antibody emicizumab. J Thromb Haemost. 2018;16(6):1078-1088.
15. Kanda Y. Investigation of the freely available easy-to-use software ‘EZR’ for medical statistics. Bone Marrow Transplant. 2013;48(3):452-458.
16. Kitazawa T, Esaki K, Tachibana T, et al. Factor VIIIa-mimetic cofactor activity of a bispecific antibody to factors IXa/IXa and X/Xa, emicizumab, depends on its ability to bridge the antigens. Thromb Haemostasis. 2017;117(7):1348-1357.
17. Mahlangu J, Oldenburg J, Paz-Priel I, et al. Emicizumab prophylaxis in patients Who have hemophilia A without inhibitors. N Engl J Med. 2018;379(9):811-822.
18. Oldenburg J, Mahlangu JN, Kim B, et al. Emicizumab prophylaxis in hemophilia A with inhibitors. N Engl J Med. 2017;377(9):809-818.
19. van Dijk K, van der Bom JG, Lenting PJ, et al. Factor VIII half-life and clinical phenotype of severe hemophilia A. Haematologica. 2005;90(4):494-498.
20. Muto A, Yoshihashi K, Takeda M, et al. Anti-factor IXa/X bispecific antibody (ACE910): hemostatic potency against ongoing bleeds in a hemophilia A model and the possibility of routine supplementation. J Thromb Haemost. 2014;12(2):206-213.
21. Hemker HC, Giesen P, Al Dieri R, et al. Calibrated automated thrombin generation measurement in clotting plasma. Pathophysiol Haemost Thromb. 2003;33(1):4-15.
22. Yada K, Nogami K, Ogiwara K, et al. Global coagulation function assessed by rotational thromboelastometry predicts coagulation-steady state in individual hemophilia A patients receiving emicizumab prophylaxis. Int J Hematol. 2019;110(4):419-430.
23. Furukawa S, Nogami K, Shimonishi N, Nakajima Y, Matsumoto T, Shima M. Prediction of the haemostatic effects of bypassing therapy using comprehensive coagulation assays in emicizumab prophylaxis-treated haemophilia A patients with inhibitors. *Br J Haematol.* 2020;190(5):727-735.

24. Schmitt C, Adamkewicz JI, Xu J, et al. Pharmacokinetics and pharmacodynamics of emicizumab in persons with hemophilia A with factor VIII inhibitors: HAVEN 1 study. *Thromb Haemostasis.* 2021;121(3):351-360.

25. Kempton CL, White GC. 2nd. How we treat a hemophilia A patient with a factor VIII inhibitor. *Blood.* 2009;113(1):11-17.

26. Malkan UY, Aksu S. Combination of novoseven and feiba in hemophiliac patients with inhibitors. *Open medicine (Warsaw, Poland).* 2018;13:618-621.