Nishiya K, Tanaka I, Yoshikawa Y, Shima M　XXII Congress of the International Society on Thrombosis and Haemostasis　7月27日　Kyoto

19)Procoagulant effect of tranexamic acid with minimal urokinase on exvivo human hemophilia model under blood flow　Ogiwara K, Nogami K, Hosokawa K　Matsumoto T, Shima M　XXII Congress of the International Society on Thrombosis and Haemostasis　7月27日　Kyoto

20)Mild clinical phenotype in a severe hemophilia a with age178his substitution associated with increased factor Xa generation　Yada K, Nogami K, Ogiwara K, Shibata M, Shima M　XXII Congress of the International Society on Thrombosis and Haemostasis　7月28日　Kyoto

21)A novel missense mutation of factor V(factor V nara:W1920R)manifested thrombosis and demonstrated a positive rezult of activavated protein C resistance assay　Shinozawa K, Nogami K, Ogiwara K, Matsumoto T, Amano K, Shima M, Fukutake K　XXII Congress of the International Society on Thrombosis and Haemostasis　7月28日　Kyoto

22)Dynamics of plasma factor VII during the continuous infusion factor VII concentrates in twenty patient with hemophilia A　Nishiya K, Nogami K, Tanaka I, Shibata M, Ogiwara K, Matsumoto T, Shima M　XXII Congress of the International Society on Thrombosis and Haemostasis　7月28日　Kyoto

23)活性化プロテインC抵抗性の新規凝固第V因子分子異常症（W1920R)の凝血学的特性と抗凝固療法の確立　荻原　建一，野上　恵嗣，篠澤　圭子，松本　智子，古川　晶子，西屋　克己，福武　勝幸，嶋　緑倫　第73回　日本血液学会　10月16日　名古屋市

24)自己血管内皮前駆細胞移植による血友病Aインヒビターに対する新規免疫寛容導入療法　松井　英人，リリクラップ　デービット，杉本　充彦，嶋　緑倫　第73回　日本血液学会　10月16日　名古屋市

25)The first national survey of thrombotic disorders in Japanese children　Ishiguro A , Taki M , Manabe A , Ogawa C , Nakadate H , Shima M　Division of Hematology,National Center for Child Health and Development
第73回　日本血液学会　10月16日　名古屋市

26)Evaluation of coagulation function on the clinical phenotype with acquired FV inhibitor patients　Matsumoto T , Nogami K , Ogiwara K , Yada K , Shima M　第73回　日本血液学会　10月14日
名古屋市

27)Effects of anti-FVIII inhibitors on the FVIII neutralization for hemophilia A with inhibitor　Yada K , Nogami K , Ogiwara K , Shima M　第73回　日本血液学会　10月14日　名古屋市

28)血友病Aインヒビター保有患児における第Ⅷ因子製剤によるインヒビター中和/持続輸注療法の凝血学的検討　西屋　克己，柴田　優，野上　恵嗣，荻原　建一，田中　一郎，松本　智子，嶋　緑倫　第53回　日本小児血液・がん学会　第9回　日本小児がん看護学会　11月25日　前橋市

29)第Ⅷ因子欠乏型出血症状であると凝血学的に診断できたtype 3 von Willebrand病の1例　古川　晶子，荻原　建一，野上　恵嗣，細川　和也，松本　智子，西屋　克己，嶋　緑倫　第53回　日本小児血液・がん学会　第9回　日本小児がん看護学会　11月25日　前橋市

30)A novel mechanism of Enhancing the Haemostatic Effect in the Combination with Recombinant Factor VIII and Activated Prothrombin Complex Concentrate(APCC) in Hemophilia A Patients with Inhibitor　Yada K , Nogami K , Ogiwara K , Shima M　American Society of Hematology　12月10日　San Diego

31)"Evaluation of Comprehensive Hemostatic Function of Patients with Von Willebrand Disease (VWD) Under Flow Using a New Microchip Flow Chamber System　Ogiwara K , Hosokawa K , Nogami K , Matsumoto T , Shima M, Nishiya K , Tanaka I , Nogami K , Ogiwara K , Yada K , Matsumoto T　American Society of Hematology　12月11日　San Diego

32)Mechanism of the Potent Activated Protein C Resistance of Novel Factor V Mutation with W1920R (FV Nara) Relative to R506Q (FV Leiden)　Ogiwara K , Shinozawa K,

Nogami K , Matsumoto T , Nishiya K , Tsujii N , Yada K , Fukutake K , Shima M American Society of Hematology　12 月 12 日　San Diego

33)Pharmacokinetics of Continuous Infusion Therapy of Factor VIII Concentrates in Hemophilia A Patients with Inhibitors
Nishiya K , Tanaka I , Nogami K , Ogiwara K , Yada K , Matsumoto T , Shima M American Society of Hematology　12 月 12 日 San Diego

34)Evaluation of Comprehensive Hemostatic Function of Patients with Von Willebrand Disease (VWD) Under Flow Using a New Microchip Flow Chamber System
Ogiwara K , Hosokawa K , Nogami K , Matsumoto T , Shima M　American Society of Hematology　12 月 13 日　San Diego

（発表誌名巻号・頁・発行年等も記入）

H．知的財産権の出願・登録状況
（予定を含む。）

1. 特許取得
特許 4671823　血液凝固因子の不活性化及び血液凝固因子

2. 実用新案登録
なし

3. その他
なし

Ⅳ. 班会議

厚生労働科学研究費補助金（難治性疾患克服研究事業）
「後天性血友病 XIII(13)の実態調査、発症機序の解明と治療方法の開発」
平成 23 年度　第 1 回班会議プログラム

日時：　平成 23 年 6 月 12 日(日)　10:00-15:00
場所：　東京国際フォーラム　会議室 G402（東京都千代田区丸の内三丁目 5-1）

09:50-10:00　受付

10:00-11:00　**22 年度報告(まとめ)と 23 年度の実施計画(全体)**　一瀬 白帝

11:00-11:05　**23 年度の実施計画(研究分担者)**
・内科・小児科サブグループ
前田 美穂 先生（日本医科大学）

11:05-12:05　**症例検討会（各 10 分）**
・村田 幸平 先生（市立吹田市民病院）
・内海 英貴 先生（群馬大学医学部）
・和田 秀穂 先生（岡山・川崎医科大学）
・甲斐 憲治 先生（広島・福山市民病院）
・杉山 裕之 先生（大阪・野江病院）
・竹迫 倫太郎 先生（岡山・水島中央病院）

(12:05-13:00)　昼食

13:00-15:00　**23 年度の実施計画(研究分担者)**　*研究分担者代理
(13:00-13:30)　**・分子病態サブグループ（各 5 分）**
惣宇利 正善（山形大学医学部）
山本 正雅 先生（奥羽大学薬学部）
*坂田 洋一 先生（自治医科大学）　*窓岩 清治 先生
矢冨　裕 先生（東京大学医学部）
丸山 征郎 先生（鹿児島大学医学部）
討論

(13:30-13:45)　**・外科・救急サブグループ（各 5 分）**
村田 幸平 先生（市立吹田市民病院）
小林 隆夫 先生（浜松医療センター）
討論

(13:45-14:20)　**・内科・小児科サブグループ（各 5 分）**
家子 正裕 先生（北海道医療大学）
*川杉 和夫 先生（帝京大学医学部）　*山本　義 先生
花房 規男 先生（東京大学医学部）
石田 文宏 先生（信州大学医学部）
和田 英夫 先生（三重大学医学部）
*嶋　緑倫 先生（奈良県立医科大学）　*西屋 克己 先生
討論

14:20-15:00　**全体討論**　一瀬 白帝

厚生労働科学研究費補助金（難治性疾患克服研究事業）
「後天性血友病 XIII(13)の実態調査、発症機序の解明と治療方法の開発」
平成 23 年度　第 2 回班会議プログラム

日時：　平成 24 年 1 月 22 日(日)　10:00～15:00
場所：　東京国際フォーラム　会議室 G604（東京都千代田区丸の内三丁目 5-1）

09:50～10:00　受付

10:00～12:00　**23 年度の研究成果と研究期間全体(22-23 年度)のまとめ、自己評価**　一瀬 白帝

(12:00～12:45)　昼食

12:45～14:30　**23 年度の研究進捗状況と班研究終了後の展望**
(研究分担者)

(12:45～13:25)　**・分子病態サブグループ**
惣宇利 正善（山形大学医学部）
山本 正雅 先生（奥羽大学薬学部）
坂田 洋一 先生（自治医科大学）
矢冨　裕 先生（東京大学医学部）
丸山 征郎 先生（鹿児島大学医学部）

(13:25～14:15)　**・内科・小児科サブグループ**
家子 正裕 先生（北海道医療大学）
川杉 和夫 先生（帝京大学医学部）
前田 美穂 先生（日本医科大学）
花房 規男 先生（東京大学医学部）
*石田 文宏 先生（信州大学医学部）　*代読
和田 英夫 先生（三重大学医学部）
嶋　緑倫 先生（奈良県立医科大学）

(14:15～14:30)　**・外科・救急サブグループ**
小林 隆夫 先生（浜松医療センター）
村田 幸平 先生（市立吹田市民病院）

14:30～15:00　**総合討論**　一瀬 白帝
その他

Ⅴ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
	なし						

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Fujii N, Souri M, Ichinose A.	A short half-life of the administered factor XIII (FXIII) concentrates after the first replacement therapy in a newborn with severe congenital FXIII deficiency.	Thromb Haemost.	107(3)	in press [Epub ahead of print]	2012
Ichinose A, Souri M.	Reduced difference of α(2)-plasmin inhibitor levels between plasma and serum in patients with severe factor XIII deficiency, including autoimmune hemorrhaphilia due to anti-factor XIII antibodies.	Int J Hematol.	95(1)	47-50	2012
Ichinose A.	Hemorrhagic acquired factor XIII (13) deficiency and acquired hemorrhaphilia 13 revisited.	Semin Thromb Hemost.	37(4)	382-8.	2011

A short half-life of the administered factor XIII (FXIII) concentrates after the first replacement therapy in a newborn with severe congenital FXIII deficiency

Noriko Fujii[1]; Masayoshi Souri[2]; Akitada Ichinose[2]

[1]Department of Pediatrics, Fukuchiyama City Hospital, Fukuchiyama, Japan;[2]Department of Molecular Patho-Biochemistry and Patho-Biology, Yamagata University School of Medicine, Yamagata, Japan

Dir Sirs,

Factor XIII (FXIII) is a fibrin-stabilising factor which crosslinks fibrin monomers among themselves as well as to α_2-plasmin inhibitor and fibronectin, and thus contributes to haemostasis, wound healing, and maintenance of pregnancy (1–3).

Congenital FXIII deficiency is a rare haemorrhagic disorder. Umbilical bleeding in the neonatal period is characteristic and the most frequent symptom (4, 5). Intracranial haemorrhage is less frequent but the leading cause of death at all ages. Plasma-derived FXIII concentrates are available for the treatment of congenital FXIII deficiency. The response to infused FXIII is mostly excellent to get a good control of bleeding (6). Regular replacement therapy with FXIII concentrates is recommended for prophylaxis of bleeding (6–9). However, an appropriate interval of FXIII administration is not known in the neonatal period, because to our best knowledge no report has ever been published on the half-life of FXIII during this stage of a patient's lifetime.

Here, we report that the half-life of the administered FXIII concentrates was markedly shortened in a male neonate with severe congenital FXIII deficiency.

A Japanese male baby was born after 36 weeks and six days of gestation with a birth weight of 2,446g by normal vaginal delivery. He has no family history of bleeding disorders, and his parents are non-consanguineous. He had hypoglycaemia after birth and received intravenous drip infusion of glucose. He had no problems with haemostasis after venipuncture such as injection and blood collection. However, excessive umbilical bleeding occurred on day 5. Umbilical bleeding stopped temporally after applying pressure, $AgNO_3$, or suturing, but, every time, a large amount of blood was seen on a covering gauze within 12–24 hours after haemostasis. Blood clots were gelatinous and fragile. There were oozing without application of pressure.

Laboratory examinations on day 5 revealed that platelet count, prothrombin time, activated partial thromboplastin time, fibrinogen, fibrinogen/fibrin degradation products, antithrombin, factors VIII, IX, and von Willebrand factor were within the normal ranges (see ▶Suppl. Table 1, available online at www.thrombosis-online.com).

Umbilical stump bleeding recurred intermittently (▶Fig. 1), and the patient developed severe anaemia (haemoglobin; Hb, 5.8 g/dl), and was thus transfused with red blood cell concentrate at 10 ml/kg on day 15. His FXIII activity was only 4% on day 12. Accordingly, he was diagnosed to have FXIII-A deficiency, which was confirmed by an amine incorporation assay, ELISA, and fibrin-crosslinking test (data not shown). In addition, Western blot analysis showed virtually no FXIII-A antigen in the patient's plasma (see ▶Suppl. Fig. 1, available online at www.thrombosis-online.com). Dot blot analyses using recombinant FXIII-A and FXIII-B (10) demonstrated negative results for anti-FXIII antibodies (data not shown).

He was injected with FXIII concentrates at 80 U/kg on day 15 immediately after receiving the FXIII result. His umbilical bleeding stopped promptly. Thereafter, he did not show any sign of bleeding, judging by any measure including magnetic reson-

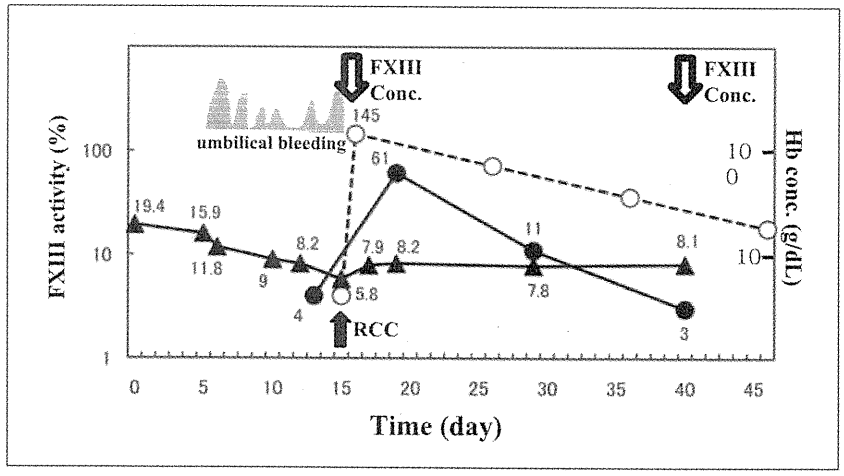

Figure 1: Clinical course of and FXIII levels in the proband. Gray-shadowed peaks (top left) show the severity/degree of umbilical bleeding. Solid circles indicate actual FXIII activities (% of normal), while open circles show theoretically calculated FXIII activities (% of normal) after the injection of FXIII concentrates (FXIII Conc.) on day 15. Hb levels (Hb conc. in g/dl) are depicted by solid triangles. Open arrows indicate the administration of FXIII concentrates at 240 U. Both FXIII activity and Hb conc. are shown on the logarithmic scale. A small arrow stands for the infusion of red blood cell concentrate (RCC). Numbers next to markers represent values of FXIII activities and Hb levels.

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Thromb Haemost 2012; 107: ■■■

ance imaging, ultrasonography as well as extensive physical inspection and examinations.

On day 40 (four weeks after the first replacement therapy), his FXIII activity decreased again to less than 3% (less than the detection limit of a commercial ammonia release assay; Berichrome FXIII, Dade Behring AG, Marburg, Germany). Therefore, a half-life of the administered FXIII in the patient was estimated to be as short as about four days after the first replacement therapy using plasma-derived FXIII concentrates (▶Fig. 1). Accordingly, thereafter the patient was started on regularl FXIII concentrate application at 12.5 U/kg every three weeks. No bleeding has occurred in the 15 months since then. He kept a trough of FXIII activity at 4% about six months after birth. It is noteworthy that his FXIII activity increased to 14% by a dose of 25 U/kg three-weeks after the last prophylactic replacement at 15 months of age, suggesting that a half-life of the administered FXIII became longer.

A family study revealed that his father's and mother's FXIII activities were 48% and 38% of normal, respectively. An uncle and a grandfather of the father's side as well as a grandmother of the mother's side had moderately reduced FXIII activities (see ▶Suppl. Fig. 2, available online at www.thrombosis-online.com), suggesting that they are all heterozygotes of FXIII deficiency.

Gene sequencing analyses and genetic diagnoses of *F13A* confirmed that the proband was a compound heterozygote of Tyr204Stop and Ser708Arg mutations, and that family members of his father's and mother's sides have Tyr204Stop and Ser708Arg, respectively. These mutations likely bring about structural changes in the variant FXIII-A molecules (paper in preparation by MS). Recently a German group has stated that heterozygotes of FXIII deficiency can manifest bleeding symptoms upon various stress/challenge, such as trauma and major surgery (5). In contrast, none of the carriers of the two mutations in this patient's family showed excessive bleeding, suggesting that they did not have such stresses/challenges and/or that these mutations may cause only minor haemostatic defects.

Neonates are physiologically in an enhanced fibrinolytic state, because anti-fibrinolytic ability decreases and bleeding symptoms occur at a high rate especially when FXIII activity decreases (11–13). Therefore, patients with congenital FXIII deficiency usually develop umbilical bleeding during the neonatal period (4, 5). It is important to diagnose such patients early enough, in order to achieve haemostasis immediately by urgent supplement with FXIII concentrates. This is also true for early diagnosis and early prophylaxis (14).

Prophylactic therapy is recommended using plasma-derived FXIII concentrates at a dose of 10–20 U/kg every 4–6 weeks (4, 9, 15, 16). This long injection interval for adult patients (4, 6, 8, 17, 18) is based on the half-life of plasma-derived FXIII, about 10 days (17–19). There has not been any report on the half-life of FXIII in a neonate, which could be used as a basis for a dose and an interval of FXIII replacement therapy for neonatal FXIII deficiency. Nevertheless, we needed to inject FXIII concentrates every three weeks because the half-life of the administered FXIII was very short in our neonate patient. It is consistent with the fact that dosage regimens vary widely depending upon patient's response and pharmacokinetics (16).

Therefore, this novel finding will contribute to the consideration of an optimal regimen of FXIII substitution for a new neonate case of this disease. This is consistent with a general concept that the rational interval of replacement therapy must be relevant to the half-life of each drug.

In summary, we propose that determination of the half-life of FXIII in neonatal cases with congenital FXIII deficiency is important for physicians to decide an interval of replacement therapy with FXIII concentrates individually for each neonate case. This may be also applied to recombinant FXIII-A products (20). FXIII supplementation may increase clot firmness (21).

Acknowledgements

The authors thank Prof. M. Shima of Nara Medical College for management of the patient after he moved to Nara Prefecture, and Ms. L. Boba for her assistance in preparation of the manuscript. This study was supported in part by the research grant from the Japanese Ministry for Health, Welfare, and Labor. This study was presented in part at the 23rd International Society on Thrombosis and Haemostasis meetings in Kyoto in July 2011. Written informed consent was obtained from the newborn patient's parents as well as from all participants in the family study.

Conflict of interests

None declared.

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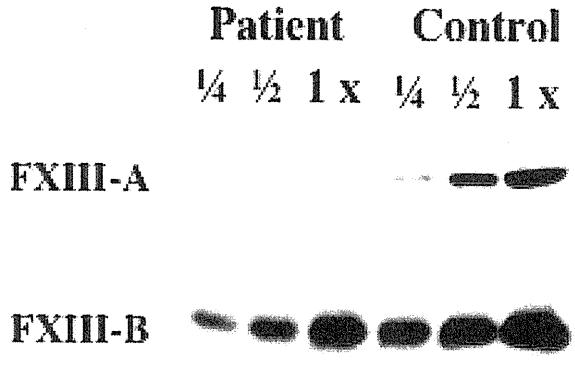
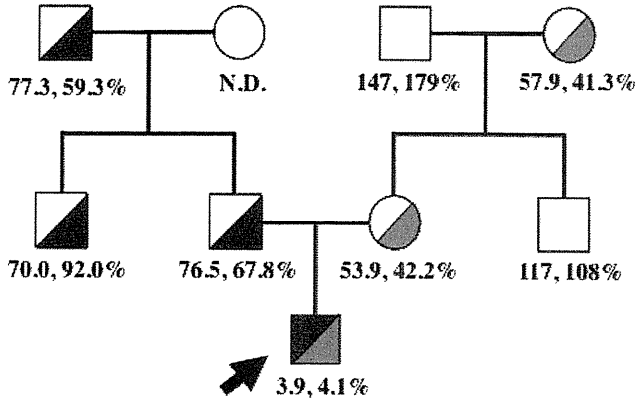
Supplementary Material to Fujii et al. "A short half-life of the administered factor XIII (FXIII) concentrates after the first replacement therapy in a newborn with severe congenital FXIII deficiency" (Thromb Haemost 2012; 107.3)



Suppl. Figure 1: FXIII-A and FXIII-B antigens in the proband's plasma. Essentially no FXIII-A antigen was detected by Western blotting, while FXIII-B protein was found to have decreased mildly in the patient (left) when compared to a normal control (right). An anti-FXIII-A antibody was rabbit polyclonal and homemade as described previously (Ichinose et al, Biochemistry 25; 4633-4638, 1986). An anti-FXIII-B antibody (RAHu/FXIII-S; polyclonal rabbit antiserum) was purchased from NORDIC immunological Laboratories (Tilburg, The Netherlands).



Suppl. Figure 2: Family pedigree and FXIII activity and FXIII-A antigen levels.
An arrow indicates the proband of severe FXIII deficiency in this family. FXIII activities and antigen levels are determined by an amine incorporation assay and ELISA, respectively. FXIII activities (% of normal) are followed by FXIII antigen levels (% of normal). Blue-fills and pink-fills stand for Tyr204Stop and Ser708Arg mutations, respectively. N.D.; not determined because a sample was not available.

Suppl. Table 1: Laboratory tests (day 5).

WBC	9,550	/μL	TP	4.5	g/dL
RBC	341×10^4	/μL	T-bil	12.4	mg/dL
Hb	11.8	g/dL	GOT	23	IU/L
Ht	34.7	%	GPT	7	IU/L
Plt	22.5×10^4	/μL	LDH	358	IU/L
			CK	129	IU/L
PT	9.9	sec	BUN	3	mg/dL
PT-INR	0.90		Cre	0.40	mg/dL
aPTT	48.6	sec	Na	145	mEq/L
Fibrinogen	121	mg/dL	K	4.2	mEq/L
FDP	3.1	μg/mL	Ca	9.0	mg/dL
Antithrombin	54.6	%			
F /8 act.	104.6	%	CRP	0.02	mg/dL
F /9 act.	25.7	%			
vWF	173	%			

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RAPID COMMUNICATION

Reduced difference of α_2-plasmin inhibitor levels between plasma and serum in patients with severe factor XIII deficiency, including autoimmune hemorrhaphilia due to anti-factor XIII antibodies

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Abstract Coagulation factor XIII/13 (FXIII/13) stabilizes fibrin molecules by creating crosslinks with other fibrin molecules as well as with α_2-plasmin inhibitor (α_2-PI). "Hemorrhagic acquired FXIII/13 deficiency" was formerly considered rare, but has been increasing recently in Japan. During the 10 months of our nationwide campaign, we diagnosed five new patients with "acquired hemorrhaphilia due to anti-FXIII/13 autoantibodies," after examining 20 newly suspected cases of "hemorrhagic acquired FXIII/13 deficiency." When FXIII/13 activity was reduced to less than 50% of normal, it was proportional to the difference in α_2-PI levels between plasma and serum (plasma–serum α_2-PI), likely due to its cross-linking to fibrin by activated FXIII/13. Accordingly, decreased amounts of the plasma–serum α_2-PI ex vivo may reflect reduced FXIII/13 activity in vivo. The plasma–serum α_2-PI may thus also be a useful diagnostic marker for severe FXIII/13 deficiency.

Keywords Nationwide study · Bleeding disorder · Acquired deficiency · Autoantibodies · Protein cross-linking · Anti-fibrinolytic potency

Introduction

"Acquired hemophilia (AH)[1]" is an autoimmune disease resulting from the presence of autoantibodies directed against clotting factors [1], most commonly factor VIII (FVIII) [2], less commonly factors IX, V, VII, and XI, and rarely factor XIII (FXIII, or FXIII/13 to avoid confusion with FVIII and FXII[2]) and prothrombin [3, 4]. Recently, recognition of this bleeding disorder has been on the rise; e.g., the incidence of acquired hemophilia-A due to anti-FVIII inhibitors has been estimated at 1.5 cases per one million population per year [5].

In contrast, information on only a small number of cases of "acquired/autoimmune hemorrhaphilia (see footnote 1) due to anti-FXIII/13 inhibitors (AH-13)" has been collected [6, 7]. However, AH-13 has also recently been on the increase in Japan (27 cases at the time of submission; unpublished data). AH-13 must be distinguished from regular "hemorrhagic acquired FXIII/13 deficiency (HA-FXIII/13def)" [6], as AH-13 tends to be more severe than

On behalf of the Japanese collaborative research group on "Acquired hemo(rrha)philia due to factor XIII/13 deficiency".

Members of the Japanese collaborative research group include: Ichinose A, Souri M, Iwata H, Sakata Y, Yatomi Y, Maruyama I, Kawamae K, Shigematsu H, Kobayashi T, Murata K, Ikeda M, Yukawa M, Sugita K, Maeda M, Kawasugi, K, Ishida F, Matsushita T, Shima M, Shirahata A, Madoiwa S, Fukutake K, Kitajima I, Takamatsu J, Miyata S, Fujii T, Takano K, Nakao A, Eguchi Y, Sakon K, Ojiro M, Ieko M, Tamai Y, Matsuura Y, Taki M, Wada H, Higasa S, and Nishikawa T.

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[1] Acquired hemophilia is not an official naming but is a tentative, working name for this category of diseases, because it is not included in the current version of the WHO ICD (2007). "Acquired hemorrhaphilia" seems to be a more logical and proper naming.

[2] Occurring frequently in clinical fields and less commonly in scientific fields even in the official journal of the International Society of Thrombosis and Hemostasis as well as in PubMed.

regular HAFXIII/13def, and requires immunosuppressive therapy to eradicate autoantibodies, together with FXIII/13 replacement therapy to stop bleeding.

Nevertheless, even severe FXIII/13 deficiency can be overlooked by physicians as there is no routine coagulation test to determine FXIII/13 activity. Thus, in the present study, we explored the possibility that the difference in α_2-PI concentrations between plasma and serum (plasma–serum α_2-PI) is a reliable indicator of FXIII/13 activity.

Methods

The authors have been called in as consultants for many AH-13 cases in recent years, so we embarked on a nationwide campaign concerning this disorder in Japan in 2009. A flyer and a simple questionnaire on past cases of HAFXIII/13def [6] were sent to 1,757 university or public hospitals and hematologists. From August 2009 to June 2010, patients who were bleeding actively due to unknown causes were recruited into the study and examined. Inclusion criteria were as follows [6, 7]: when otherwise healthy subjects suddenly manifested severe bleeding symptoms in the absence of a family history, prolonged clotting times, or platelet abnormalities, their physicians called members of the study group (see title page) into consultation. This study was approved by The Institutional Review Board of the Yamagata University School of Medicine. All procedures were conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all individuals.

Whole blood of patients was collected into 1/10 volume of 3.8% sodium citrate as an anticoagulant and centrifuged at 1,000*g* for 10 min at 4°C to prepare plasma. Serum was prepared separately by collecting blood into a Venoject II tube (Terumo, Tokyo, Japan) containing polyester film coated with glass microparticles to enhance coagulation. Plasma and serum samples were quick-frozen and sent to a commercial laboratory (SRL Ltd., Hachioji, Japan) to measure plasma FXIII/13 activity (normal range 70–140%) by ammonia release assay (Berichrom FXIII, Dade Behring, Marburg, Germany) as well as plasma and serum levels of α_2-PI using a commercial kit (Testzyme S APL, Sekisui Medical, Tokyo, Japan; normal range 80–130% for plasma; coefficient of variation: <5%). The amount of plasma–serum α_2-PI was calculated by subtracting the serum concentration of α_2-PI from an adjusted plasma concentration of α_2-PI (divided by 0.9 to allow for 1:9 dilution with the anticoagulant). The ratio of plasma–serum α_2-PI was calculated by dividing the amount of plasma–serum α_2-PI by the amount of the adjusted plasma concentration of α_2-PI.

For statistical analysis, comparison between groups by the Mann–Whitney and Kruskal–Wallis test, and correlation between parameters by the Spearman's coefficient were carried out using the software program JMP ver. 6.0.3 (SAS Institute, Cary, NC, USA), and differences were determined to be statistically significant at a P value of <0.05.

Results and discussion

We diagnosed five new AH-13 patients over a 10-month period, when we examined 20 newly suspected cases with HAFXIII/13def; 15 of these cases were confirmed to be persistent/chronic HAFXIII/13def (range of FXIII/13 activity, 5–66%). The remaining five cases showed normal FXIII/13 activity at the time of examination, thus they were diagnosed as having transient FXIII/13 deficiency. We then further confirmed by our laboratory tests that five of the 15 cases with HAFXIII/13def were AH-13, as determined by a mixing assay using the amine incorporation method [8] and an immunoblot test for antibodies against recombinant FXIII/13-A and recombinant FXIII/13-B (in preparation). The AH-13 cases showed greatly reduced FXIII/13 activity (<25% of normal), four of which were found to be idiopathic and one of which had an abdominal aortic aneurysm. One of the remaining 10 cases with HAFXIII/13def had no underlying disease, while others had a thoracic or abdominal aortic aneurysm, esophageal, uterine or rectal cancer, soft tissue tumor, hepatitis, pneumonia, or rheumatoid arthritis.

The average activity level of FXIII/13 was significantly lower in the 20 new cases suspected of being HAFXIII/13def, compared with 20 normal controls (mean and standard deviation of FXIII/13 activity, 45.5 ± 30.7 vs. $96.6 \pm 17.7\%$; $P < 0.0001$; Fig. 1a). This is very likely due to its over-consumption in most cases and to anti-FXIII/13 autoantibodies at least in five AH-13 cases. The possibility of FXIII/13 hypo-biosynthesis, however, cannot be completely excluded.

Plasma concentrations of α_2-PI were slightly lower than in normal controls (94.8 ± 26.2 vs. $112.7 \pm 11.1\%$; $P = 0.01$; Fig. 1b). Three of four patients with reduced α_2-PI levels showed plasmin/α_2-PI complex levels higher than normal (data not shown), suggesting that α_2-PI was, at least in part, consumed by plasmin.

Notably, the difference in α_2-PI levels between plasma and serum (plasma–serum α_2-PI) was also significantly lower in the HAFXIII/13def-suspected cases compared with normal controls (9.1 ± 8.8 vs. $18.9 \pm 5.8\%$, respectively; $P < 0.001$; Fig. 2a). The ratio of plasma–serum α_2-PI to plasma α_2-PI was then calculated, since plasma concentrations of α_2-PI varied and were slightly lower than normal controls (94.8 ± 26.2 vs. $112.7 \pm 11.1\%$; $P = 0.01$); the ratio to the adjusted plasma α_2-PI was again

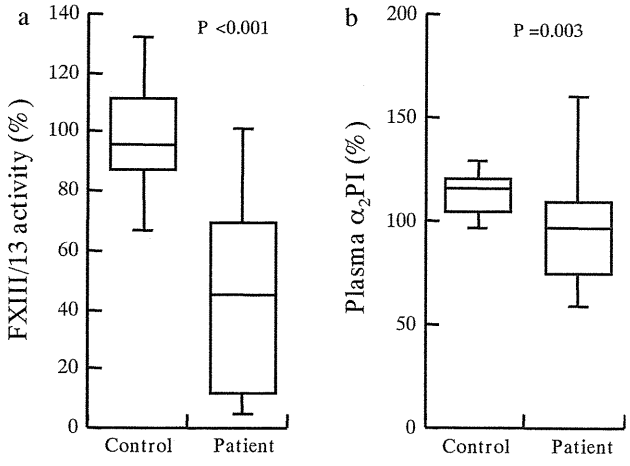

Fig. 1 Comparison of F13 activity (**a**) and α_2-PI (**b**) between HAF13def-suspected patients and normal controls. F13 activity and α_2-PI concentrations in plasma were measured and compared statistically among 20 suspected cases with HAF13def and 20 normal individuals, as described in the "Methods". *Box plots* represent median, quartiles, and range of laboratory measurements

significantly lower in the HAFXIII/13def-suspected cases compared with normal controls (0.083 ± 0.076 vs. 0.15 ± 0.044, respectively; $P < 0.001$; Fig. 2b). The AH-13 cases, in particular, demonstrated severely reduced plasma–serum α_2-PI amounts and its ratio to plasma α_2-PI (crosses in Fig. 2a, b).

The linear relationships between these parameters, moreover, were significant among the HAFXIII/13def-suspected cases ($R^2 = 0.52$ and 0.56; $P < 0.001$ and <0.001, respectively; left in Fig. 2a, b). There was no relationship between FXIII/13 activity and the amount of plasma–serum α_2-PI and its ratio to the adjusted plasma α_2-PI among members of the normal control group ($R^2 = 0.13$ and $= 0.085$; $P = 0.16$ and 0.26, respectively; right in Fig. 2a, b).

These results indicate that low levels of plasma–serum α_2-PI and its ratio to the adjusted plasma α_2-PI may reflect reduced FXIII/13 activity, especially below 50%, i.e., in evident HAFXIII/13def cases, which include AH-13 cases. FXIII/13 levels at more than 50% of normal may be sufficient to achieve the plateau amount of plasma–serum α_2-PI, i.e., approximately 20% of plasma α_2-PI which coincides with the plateau level of crosslinked α_2-PI by activated FXIII/13 (FXIII/13a) [9, 10]. It is highly likely that most of the plasma–serum α_2-PI molecules represent those of α_2-PI that have covalently ligated to blood clots. A subset of plasma α_2-PI may have lost its crosslinking ability as an FXIII/13a substrate for unknown reasons in the HAFXIII/13def cases.

Amounts of plasma–serum α_2-PI reflect ex vivo FXIII/13 activity against natural substrates. It is very likely that in severe HAFXIII/13def cases, amounts of crosslinked α_2-PI that have covalently ligated to hemostatic clots are reduced in vivo, as well. This may, in turn, lead to decreased resistance to fibrinolysis in a hemostatic clot, and its premature lysis [11]. It has been reported that model thrombi from an FXIII/13-deficient patient lysed more quickly than normal thrombi; replacement therapy with FXIII/13 concentrate normalized lysis at about 50% FXIII/13 activity in plasma [12]. Complete stabilization of thrombi was also achieved in vitro at 0.5 U/mL FXIII/13 [13].

In addition, crosslinked α_2-PI may play a significant role in the inhibition of spontaneous lysis of a retracted clot more than in that of a non-retracted clot [11]. Consistently, we found that clot retraction of platelet–fibrin was absent in FXIII/13 A subunit-deficient mice [14] manifesting severe bleeding symptoms [15, 16].

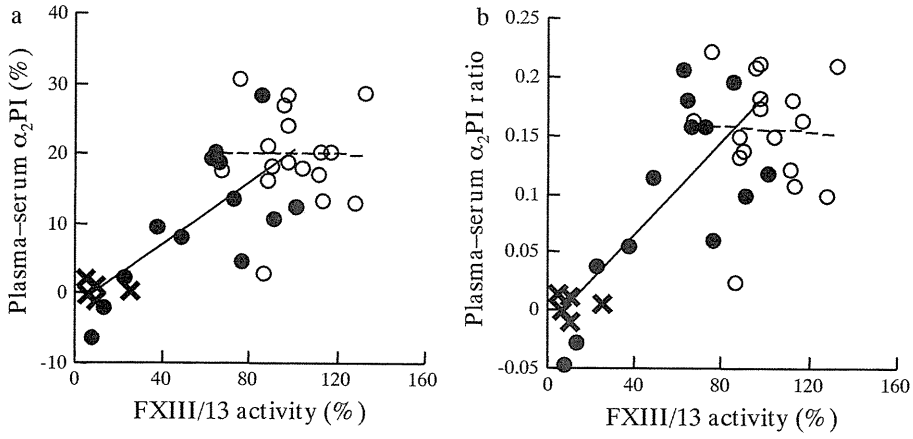

Fig. 2 Relationship between FXIII/13 activity and plasma–serum α_2-PI levels in HAFXIII/13def-suspected cases and normal controls. Correlation of F13 activity with amounts of plasma–serum α_2-PI (an adjusted plasma α_2-PI–serum α_2-PI) (**a**) and that with the plasma–serum α_2-PI ratio (the amount of plasma–serum α_2-PI/the adjusted plasma α_2-PI) (**b**) are shown in 20 suspected cases with HAF13def (*solid circles;* 5 crosses are AH13 cases) and in 20 normal individuals (*open circles*). The *solid* and *broken regression lines* are for the patients and normal individuals, respectively

In summary, we propose reduced plasma–serum α_2-PI as an indicator of significantly decreased FXIII/13 activity in patients with HAFXIII/13def including AH-13, an indicator which may contribute to quick diagnosis, and, consequently, successful treatment of severe FXIII/13 deficiency in the clinical fields.

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Conflict of interest None of the authors has any conflict of interest.

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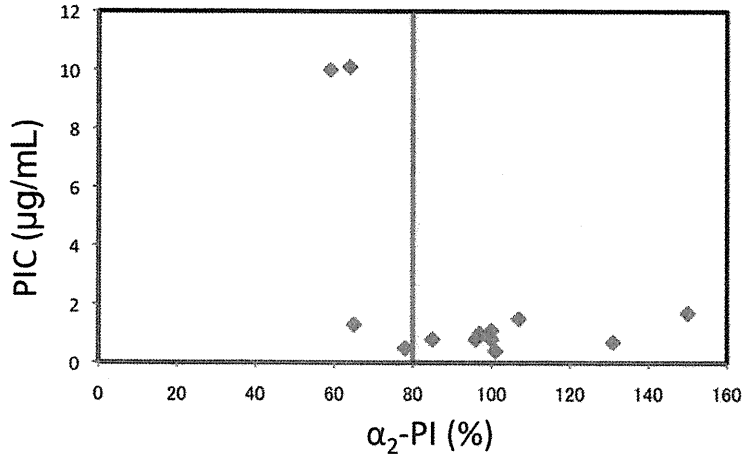
Electronic supplementary material

Below is the link to the electronic supplementary material.



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Supplementary Fig. 3 (JPEG 71.5 kb)

Footnotes

1 Acquired hemophilia is not an official naming but is a tentative, working name for this category of diseases, because it is not included in the current version of the WHO ICD (2007). "Acquired hemorrhaphilia" seems to be a more logical and proper naming.

2 Occurring frequently in clinical fields and less commonly in scientific fields even in the official journal of the International Society of Thrombosis and Hemostasis as well as in PubMed.

Hemorrhagic Acquired Factor XIII (13) Deficiency and Acquired Hemorrhaphilia 13 Revisited

Akitada Ichinose, M.D., Ph.D.[1]

ABSTRACT

Coagulation factor XIII (F13) circulates in blood as a heterotetramer composed of an A subunit dimer and a B subunit dimer. It is activated by thrombin and crosslinks fibrin monomers. Congenital F13 deficiency demonstrates a lifelong bleeding tendency, abnormal wound healing, and recurrent miscarriages, and it first manifests as umbilical bleeding after birth. In contrast, secondary F13 deficiencies due to its overconsumption and/or hypobiosynthesis by disseminated intravascular coagulation, major surgery, liver diseases, and other disorders are rather common but rarely complicated with bleeding symptoms. Recently, consultations with physicians who have patients with hemorrhagic-acquired F13 deficiency with anti-F13 inhibitors (acquired hemorrhaphilia 13) have indicated an increase in this disease in Japan. We performed a nationwide survey, supported by the Japanese Ministry of Health, Welfare and Labor and confirmed 21 Japanese cases of this disease with anti-F13 inhibitors. Because neither prolonged clotting times nor reduced platelet counts are observed in patients with this disease, many more cases may have been overlooked. Physicians must be mindful of acquired hemorrhaphilia 13 when seeing such patients and should measure F13 activity. Products containing F13 are effective for hemostasis generally, and immunosuppressive therapy must be started immediately to eradicate anti-F13 antibodies.

KEYWORDS: Acquired bleeding tendency, protein crosslinking enzyme, autoantibody, decreased production, increased consumption

THROMBOPHILIA VERSUS HEMOPHILIA

The 21st century is "the era of thrombosis" including myocardial infarction, cerebral infarction, and pulmonary thromboembolism. The number of deaths due to thrombosis is two to three times higher than that of cancer in the Western countries and is increasing more than cancer in the Eastern countries, including Japan. Thrombosis is a multifactorial disease caused by a combination of various genetic and environmental factors. The genetic tendencies predisposing to thrombosis are called *thrombophilia*; the term literally means "love of thrombus." Thrombophilia has been vigorously investigated to control and manage thrombotic complications.

In contrast, inherited bleeding disorders are mostly monogenic diseases whose causes were almost

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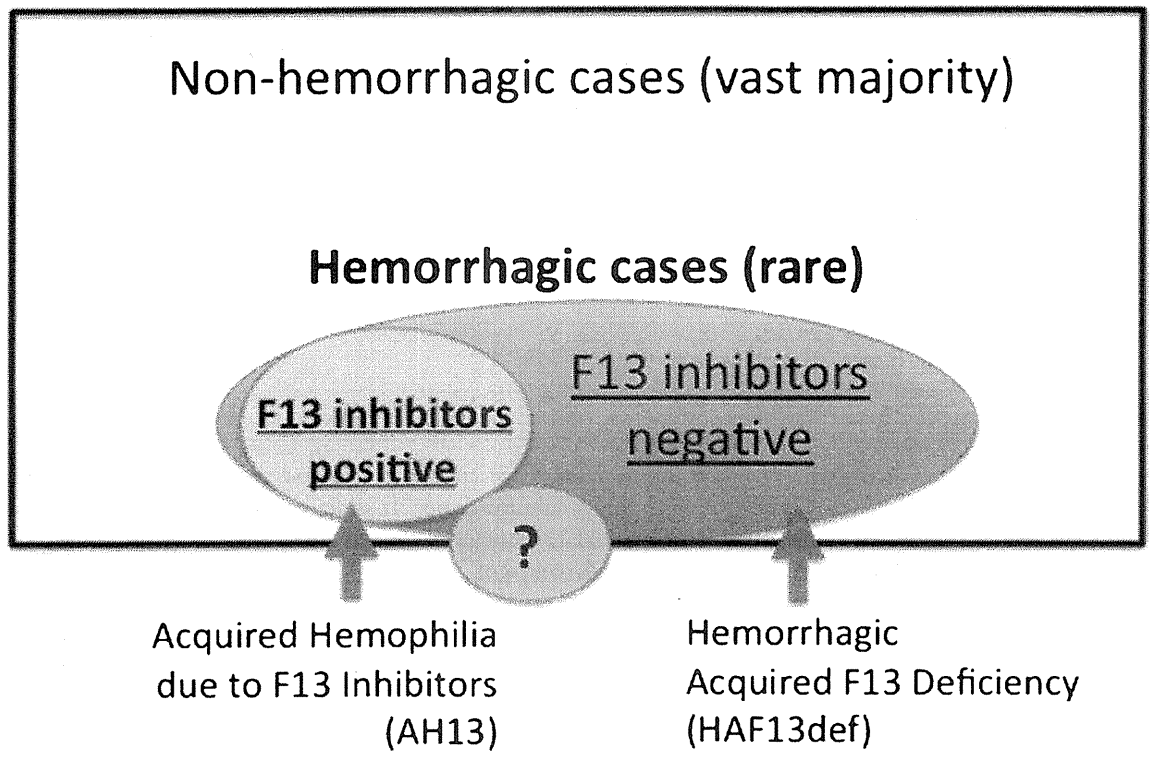

Figure 1 The concept of acquired hemophilia XIII(13)(AH13) and hemorrhagic acquired FXIII(13)deficiency (HAF13def). AH13 is caused by F13 inhibitors, while HAF13def is due to severe F13 deficiency without inhibitors. However, the diagnosis of HAF13def is tentative because of the lack of complete examination of potential cases for inhibitors against other F13-related molecules (indicated by circled question mark [?]). In contrast, the diagnosis of AH13 is definite because the presence of anti-F13 antibodies is confirmed by immunological methods.

completely solved by the late 1980s at the genetic and molecular levels. The genetic tendencies predisposing to bleeding are called *hemophilia,* "love of blood"; it may be more appropriate to called them hemorrhaphilia[1] because their core symptom appears as "love of bleeding." Hemophilia A and B are common outcomes of congenital factor (F)VIII and FIX deficiency, respectively. Hemophilia C, para-hemophilia, and pseudo-hemophilia are also caused by congenital FXI, FV, and von Willebrand factor deficiency, respectively. In contrast, acquired hemophilia (AH) is an autoimmune disease resulting from the presence of autoantibodies directed against clotting factors,[2] most commonly FVIII, less commonly FIX, FV, FVII, and FXI, and rarely FXIII (or F13 to avoid confusion with FVIII) and prothrombin. This bleeding disorder has become increasingly recognized. For example, the incidence of acquired hemophilia A (AHA) was estimated at 1.5 cases per 1 million population per year.[3]

In the case of AH due to F13 inhibitors, Lorand and Egbring et al summarized 22 cases including one congenital F13 deficiency case >15 years ago.[4,5] Its mortality rate is rather high, ~25%. Because I had been consulting on many such cases, I began a nationwide study in Japan in 2009. The epidemiology, pathophysiology, diagnosis, and management of hemorrhagic acquired F13 deficiency (HAF13def) or AH due to F13 deficiency (AH13) are described briefly in this review (Fig. 1).

FACTOR XIII (F13)

Transglutaminases (TGases) are enzymes that catalyze the formation of ε-(γ-glutamyl)lysine bonds, in so-called protein crosslinking reactions, between several proteins.[6] A total of 10 members of the TGase family were identified in the Human Genome Project. F13 is also called plasma TGase and fibrin stabilizing factor, and it circulates in blood as a heterotetramer consisting of two catalytic A subunits (F13-A) and two noncatalytic B subunits (F13-B), A_2B_2.[7] The gene organization of TGase was first established for F13-A in 1988; its gene is coded by 15 exons interrupted by 14 introns. The primary structure of TGase was also first established in 1986 for F13-A; it consists of 731 amino acid residues. F13-As of placenta and recombinant proteins have been crystallized. X-ray crystallography demonstrated that F13-A is composed of five distinct domains: an activation peptide, β-sandwich, central core (containing the active site Cys-314), barrel 1, and barrel 2 regions.