厚生労働科学研究費補助金（難治性疾患等政策研究事業（難治性疾患政策研究事業））
分担研究報告書

自己免疫性出血症治療の「均てん化」のための実態調査と「総合的」診療指針の作成に関する研究

分担研究課題　後天性 von Willebrand 症候群（AvWS）の病態に関する研究

研究分担者　松下　正　名古屋大学医学部付属病院輸血部　教授

研究要旨

Acquired von Willebrand syndrome(AvWS)は後天性に von Willebrand Factor (VWF)が低下することで出血傾向を呈する疾患であるが、その病態には不明な点が多く、診断法も確立されていない。本研究ではリストセチンコファクター活性ベースのベセスダ法による機能検査と ELISA 法による免疫学的抗原同定検査を行い、AvWS の診断法を確立することを目的としている。今年度は 3 症例において検討を行い、ELISA 法ではインヒビターを十分に同定できなかったが、ベセスダ法においては臨床所見と相関する良好な結果が得られた。

A．研究目的

Acquired von Willebrand syndrome(AvWS)は後天性に von Willebrand Factor (VWF)が低下することで出血傾向を呈する疾患である。大きくは抗 VWF 抗体の出現による免疫学的機序のものと非免疫学的な機序のものに分けられるが、特に免疫学的機序によると考えられているタイプは、いまだその病態は不明な点が多い。そのため、確定診断をする検査が確立されておらず、臨床症状などから、総合的に診断しているのが実情である。本研究では免疫学的機序によると考えられる AvWS に対して、確定診断に至ることのできる精度を持つ検査方法を確立し、AvWS の病態を解明することで至適な治療法を樹立することが目的である。

B．研究方法

診断のためセットアップしたアッセイ系は二つである。抗 VWF インビターの機能的検査（リストセチンコファクター活性ベースのベセスダ法）と抗 VWF 自己抗体の免疫学的検査(ELISA 法)である。この二つのアッセイを先天性 VWD type3 インヒビター合併症例の検体を用いてセットアップし、インヒビター検出の再現性、ならびに臨床所見との整合性において妥当であると考えられた結果を得た上で、AvWS 症例検体の測定を行った。

（倫理面への配慮）

当施設では匿名化された検体のみを受領し、検討を行った。

C．研究結果

今年度は 3 症例に対して検査を行った。その結果、いずれの症例においても、ELISA 法では再現性のあるインヒビター同定はできなかったが、ベセスダ法では、病勢に相関すると考えられるリーズナブルな結果が得られた。

D．考察

ELISA 法によるインヒビター同定は海外のグループからも報告されているように、本研究でも単独では診断に寄与するような結果を得ることはできていないため、今後も改良を続ける必要があると考えられた。ベセスダ法による機能検査においては非働化のステップを加えるなど、様々な検討を行った結果、今回検討した 3 症例では臨床症状と一致した結果が得られた。しかしながら、結果のレンジが狭く、細かな病勢評価には不向きな点もある。カットオフ

ポイントを含め、今後の検討が必要であると考えられた。

E．結論

AvWS と考えられる症例に ELISA 法による抗原同定検査とベセスダアッセイによるインヒビター機能検査を施行した。ELISA 法では臨床所見に合致した結果が得られなかったが、ベセスダアッセイでは良好な結果が得られ、今後の展望に期待が持てる結果であった。

F．健康危険情報

なし

G．研究発表

1. 論文発表

1. Kishimoto M, Suzuki N, Murata M, Ogawa M, Kanematsu T, Takagi A, Kiyoi H, Kojima T, Matsushita T.
The first case of antithrombin-resistant prothrombin Belgrade mutation in Japanese.
Ann Hematol. 2015 Oct 19. [Epub ahead of print]

2. Matsushita T.
Current progress and future direction of hemophilia treatment.
Rinsho Ketsueki. 2015 Oct;56(10):2100-9.

3. Kanematsu T, Suzuki N, Yoshida T, Kishimoto M, Aoki T, Ogawa M, Kagami Y, Kiyoi H, Matsushita T, Kunishima S.
A case of MYH9 disorders caused by a novel mutation (p.K74E).
Ann Hematol. 2015 Sep 17. [Epub ahead of print]

4. Ihara A, Suzuki N, Matsushita T, Itinose A.
[Acquired von Willebrand syndrome in a patient with immune thrombocytopenic purpura].
Rinsho Ketsueki. 2015 Jul;56(7):901-4.

2. 学会発表

1. 松下正　第 37 回日本血栓止血学会学術集会イブニングセミナー
「凝固第Ⅷ因子インヒビターとその対策」
平成 27 年 5 月 21 日（木）
甲府市総合市民会館

2. 松下正　第 77 回日本血液学会学術集会コーポレートセミナー
「薬物動態予測に基づく血友病治療」
平成 27 年 10 月 17 日（土）
ホテル金沢

H．知的財産権の出願・登録状況
（予定を含む。）

1. 特許取得
なし

2. 実用新案登録
なし

3. その他
なし

厚生労働科学研究費補助金（難治性疾患等政策研究事業（難治性疾患政策研究事業））
分担研究報告書

自己免疫性出血症治療の「均てん化」のための実態調査と「総合的」診療指針の作成に関する研究

分担研究課題　後天性線溶異常症に関する研究

研究分担者　浦野　哲盟　浜松医科大学医学部　教授

研究要旨

自己免疫性出血症の内、線溶系因子に対する自己抗体による症例の診断及び重症度分類の基盤となる、検査法の確立を目指す。具体的には「包括的線溶活性測定法」と「微量 PAI-1 活性測定法」の確率を目指す。

A．研究目的

自己免疫性出血症の内、線溶系因子に対する自己抗体による症例の診断及び重症度分類の基盤となる、検査法の確立を目指す。

B．研究方法

① 包括的線溶活性測定法の確立

前回の厚労科研時に取り組んだ「包括的線溶活性測定法」の検証を行い、線溶系因子の異常をどのように反映するか検証する。方法は、採血後の全血をトロンビン処理後、一定時間反応後に生成された D-dimer 量を測定する方法である。その際アプロチニン添加により線溶系を抑制した検体における D-dimer 量と比較することにより、各検体固有の線溶活性、並びに固有のフィブリン安定化程度を検出することができる。

② 微量 PAI-1 活性測定法の確立

AlphaLISA 法を用いた、微量 PAI-1 抗原量、並びに活性型 PAI-1 測定法を本学薬理学講座の岩城孝行准教授と共同で開発する。抗原量に関しては、通常の ELISA 法に比べ２オーダー高い感度で測定可能である結果をすでに得ている。

③ PAI-1 中和抗体の同定及び活性測定法の確立

PAI-1 欠損症例は、euglobulin clot lysis time (ECLT) が calcium ion (Ca^{++}) 存在下で短縮するという PAI-1 依存性及びトロンビン依存性の現象を欠く事実より発見された。同法を用いて、正常血漿の患者血漿添加に伴う Ca^{++} 依存性 ECLT の短縮が患者血漿により消失する現象を用い、PAI-1 中和抗体存在のスクリーニングが可能か検討する。また②を用いて、同様に正常血漿中の活性型 PAI-1 量への PAI-1 中和抗体の影響を解析し、スクリーニング法として妥当か検討する。

（倫理面への配慮）

症例の解析においては、連結可能匿名化により検体を扱い、患者保護を徹底する。

C．研究結果

① 包括的線溶活性測定法の確立

依然健常人ボランティアの検査に限っており、患者検体は PAI-1 欠損症例１例のみの解析となっている。担当検査技師の異動に伴うもので、早急に改善したい。

② 微量 PAI-1 活性測定法の確立

AlphaLISA 法を用いた、微量 PAI-1 抗原量、並びに活性型 PAI-1 測定法を本学薬理学講座の岩城孝行准教授と共同で開発している。抗原量に関しては、通常の ELISA 法に比べ２オーダー高い感度で測定可能である結果をすでに得ている。Biotin 標識 uPA を用いて活性型 PAI-1 の良好な検量線を得た。当初準備した Biotin 標識 uPA が、活性中心 mol 濃度の測定に

は十分な高濃度でなかったため、再度標識中である。

③　PAI-1 中和抗体の同定及び活性測定法の確立

現在 PAI-1 中和抗体を用いて正常血漿を用いた ECLT（Calcium ion 存在下及び非存在下）への影響を解析しているところである。PAI-1 欠損を疑わせる ECLT 所見を有しながら遺伝子解析で異常が認められなかった２症例で自己抗体の有無を検査予定である。また現在臨床治験が進んでいる PAI-1 阻害薬の検体を用いて ECLT を測定している。

D．考察

線溶異常症の解析には現在進行中の「包括的線溶活性測定法」と「微量 PAI-1 活性測定法」が有効と考え、早期の確率を目指している。その中で後天性の異常症に関しては中和抗体の存在とその活性を解析する必要があり、「PAI-1 中和抗体の同定及び活性測定法」を中心に、その手法の確立を目指している。

E．結論

線溶異常症の同定方法の確立と、異常症を惹起する自己抗体の同定方法および活性測定方法の確立に向け努力中である。

F．健康危険情報

○○○○○○○○○○○○○○○○○○○○

（分担研究報告書には記入せずに、総括研究報告書にまとめて記入）

G．研究発表

1. 論文発表

Brzoska T, Tanaka-Murakami A, Suzuki Y, Sano H, Kanayama N, Urano T. Endogenously Generated Plasmin at the Vascular Wall Injury Site Amplifies Lysine Binding Site-Dependent Plasminogen Accumulation in Microthrombi. Plos One, 10: E0122196, 2015.

2. 学会発表

● 浦野哲盟 「血管内皮上の線溶活性発現調節機構と PAI-1 欠損による破綻」第 37 回日本血栓止血学会学術集会（日本血栓止血学会雑誌・26(2)・2015）

● 鈴木優子、佐野秀人、Tomczyc M、Brzoska T、浦野哲盟 “Assessment of plasminogen activator activity on vascular endothelial cell surface” 第77回日本血液学会学術集会（臨床血液学会雑誌・56(9)・2015）

H．知的財産権の出願・登録状況

なし

厚生労働科学研究費補助金（難治性疾患等政策研究事業（難治性疾患政策研究事業））
分担研究報告書

自己免疫性出血症治療の「均てん化」のための実態調査と「総合的」診療指針の作成に関する研究

分担研究課題　抗線溶因子抗体症例の調査と実験の実施

研究分担者　和田英夫　三重大学医学部　准教授

研究要旨

三重県における過去 8 年間の出血性疾患ならびに原因不明出血を検討した。凝固因子に対するインヒビターは高齢者や自己免疫疾患ならびに悪性腫瘍に見られた。原因がはっきりしない出血 30 例について検討すると、原因不明の出血は、年間数例か認められ、FXIII や fibrinogen などのフィブリンクロット形成段階ならびにクロット形成後の異常による出血も存在することが示唆された。

A．研究目的

過去 8 年間における、三重大学でコンサルトを受けた出血性疾患を検討した。ただし、血友病、フォンウイルブランド病、播種性血管内凝固(DIC)、血栓性血小板減少性紫斑病（TTP）などを血栓性微小血管障害（TMA）ならびに特発性血小板減少性紫斑病（ITP）は除外した。

B．研究方法

過去 8 年間に診療した出血性疾患は血友病 A 40 例、血友病 B 10 例、フォンウイルブランド病 20 例、その他の先天性凝固因子異常 12 例、ITP 50 例、DIC 86 例、TMA 20 例などであった。凝固因子に対するインヒビター例は 13 例、それ以外の原因による出血例は 29 例であった。これらの症例の、検査所見、基礎疾患、年齢などを検討した。

（倫理面への配慮）

本研究は三重大学の倫理審査委員会で研究計画の承認を受けており、研究参加者からインフォームドコンセントを得た。データーは中央値（25-75%タイル）で示す。

C．研究結果

インヒビター例の内訳は、非血友病 8 例、血友病 5 例であった。12 例が FVIII に

表出血性疾患

年齢	性	基礎疾患	出血原因
30	F	妊娠	fibrinogen 低下
23	F	妊娠	不明
30	F	分娩後	子宮弛緩出血
33	F	分娩後	不明
54	F	不明	不明
37	M	不明	血小板機能低下
30	F	不明	血小板機能低下
15	F	不明	不明
15	M	不明	血管性
43	M	大腸腺腫	不明
68	F	AAA	不明
64	M	心不全	血小板機能低下
40	M	不明	血小板機能低下
34	F	妊娠	不明
27	F	不明	不明
37	F	妊娠	fibrinogen 低下
36	F	不明	fibrinogen 低下
39	F	妊娠	血小板機能低下
38	F	妊娠	不明
66	F	不明	不明
81	M	骨腫瘍	肝不全
35	F	妊娠	不明
40	F	不明	不明
5	F	肝移植	後無 fibrinogen
50	M	不明	不明

40	F	不明	血小板機能低下
27	F	不明	不明
73	F	不明	血小板機能低下
35	M	不明	不明
25	F	不明	fibrinogen 低下

AAA; 腹部大動脈瘤、後無 fibrinogen; 後天性無 fibrinogen 血症

対するインヒビターであり、1 例は FV に対するインヒビターであった。非血友病のインヒビターの基礎疾患は、自己免疫疾患 2 例、悪性腫瘍 2 例、不明 4 例であり、高齢者に多く見られた。血友病インヒビター例は殆どが重症血友病例であるが、一部軽症例にも認められた。

30 例の出血性疾患の解析（表）では、妊婦に出血が多いことから、発症年齢は 20-30 歳代に多く、性別では女性に多く見られた。基礎疾患は、不明 16 例、妊娠関連 9 例などであった。原因も不明が 15 例で最も多く、血小板機能低下 7 例、fibrinogen 低下 5 例などであった。これらの患者の FXIII 活性は、69.0% (58.0 - 92.7%)で、フィブリノゲン値は 215mg/dl (169 – 253 mg/dl)で、プラスミンインヒビター活性は 114%(96 – 117%)であった。

D. 考察

出血性疾患の頻度としては、血友病、ITP、DIC、TMA などの頻度が圧倒的に多かったが、その他の原因による出血傾向も多数認められた。インヒビターでは、基礎疾患が血友病でなくてもみられ、高齢者に多く認められた。今回の検討では、FVIII に対する抗体が圧倒的に多く、FXIII やフォンウイルブランド因子ならびに線溶系因子に対する抗体は認められていないが、見過ごされている可能性は否定できない。インヒビター以外の出血性疾患では、産婦人科疾患が多く、バイアスが高い可能性は否定できない。

出血原因は、血小板機能低下が疑われる症例も多かったが、原因不明例が最も多かった。また、フィブリノゲン低下も複数例みられ、1 例は後天性フィブリノゲン異常症が疑われた。このことから、フィブリノゲンの低下が出血に関与する症例が予想以上に多いことが推測された。FXIII 値の著明低下は見られなかったが、多くの症例で FXIII の中等度低下がみられた。FXIII の中等度低下と出血との因果関係は不明であった。

E. 結論

原因不明の出血は、年間何例か認められ、FXIII や fibrinogen などのフィブリンクロット形成段階の異常による出血も存在することが示唆された。

F. 健康危険情報

G. 研究発表

1. 論文発表

1) Kamimoto Y, Wada H, Ikejiri M, Nakatani K, Sugiyama T, Osato K, Murabayashi N, Yamada N, Matsumoto T, Ohishi K, Ishikawa H, Tomimoto H, Ito M, Ikeda T: High frequency of decreased antithrombin level in pregnant women with thrombosis. Int J Hematol. 2015; 102: 253-8
2) Kamimoto Y, Wada H, Ikejiri M, Nakatani K, Sugiyama T, Osato K, Murabayashi N, Habe K, Mizutani H, Matsumoto T, Ohishi K, Ikeda T.: Hypofibrinogenemia and the α-Fibrinogen Thr312Ala Polymorphism may be Risk Factors for Early Pregnancy Loss. Clin Appl Thromb Hemost. 2015; [Epub ahead of print]
3) Takahashi N, Usui M, Naitoh K, Wada H, Mastsui T, Kobayashi T, Matsumoto T, Uemoto S, Isaji S: Elevated Soluble Platelet Glycoprotein VI Levels in Patients After Living Donor Liver Transplantation. Clin Appl Thromb Hemost. 2015[Epub ahead of print]
4) Squizzato A, Hunt BJ, Gary T. Kinasewitz GT, Wada H, ten Cate H, Thachil J, Levi M, Vicente V, Angelo AD, Di Nisio M: Supportive management strategies for

disseminated intravascular coagulation-An international consensus, Thromb Haemost, 2015 [Epub ahead of print]
5) Aota T, Wada H, Yamashita Y, Matsumoto T, Ohishi K, Suzuki K, Imai H, Usui M, Isaji S, Katayama N.: The efficacy of the administration of recombinant human soluble thrombomodulin in patients with DIC. Int J Hematol. 2016; 103: 173-179
6) Habe K, Wada H, Matsumoto T, Ohishi K, Ikejiri M, Tsuda K, Kondo M, Kamimoto Y, Ikeda T, Katayama N, Mizutani H: Plasma ADAMTS13, von Willebrand Factor (VWF), and VWF Propeptide Profiles in Patients With Connective Tissue Diseases and Antiphospholipid Syndrome. Clin Appl Thromb Hemost. 2016 [Epub ahead of print]
7) Ota S, Wada H, Mastuda A, Ogihara Y, Yamada N, Nakamura M, Ito M: Anti-Xa activity in VTE patients treated with fondaparinux. Clin Chim Acta、2015; 442: 22-23
8) Wada H, Aota T, Matsumoto T, Suzuki K, Imai H, Katayama N: Antithrombin or thrombomodulin administration in severe pneumonia patients with sepsis and disseminated intravascular coagulation: comment on two papers. J Thromb Haemost. 2015; 13: 684-5
9) Wada H, Aota T, Yamashita Y, Matsumoto T, Katayama N: New Insights of the Tissue Factor Pathway Inhibitor in Patients with Hypercholesterolemia Treated with Statins. J Atheroscler Thromb. 2015; 22: 654-5
10) Habe K, Wada H, Matsumoto T, Ohishi K, Ikejiri M, Matsubara K, Morioka T, Kamimoto Y, Ikeda T, Katayama N, Mizutani H: Presence of antiphospholipid antibodies is a risk factor for thrombotic events in patients with connective tissue diseases and idiopathic thrombocytopenic purpura. Internal Med,(in press)
11) Yamaguchi T, Wada H, Miyazaki S, Hasegawa M, Wakabayashi H, Asanuma K, Fujimoto N, Matsumoto T, Ohishi K, Sakaguchi A, Yamada N, Ito M, Katayama N, Sudo A: Fibrin related markers for diagnosing acute or chronic venous thromboembolism in patients with major orthopedic surgery. Int J Hematol 2016 (in press)
12) Ikejiri M, Wada H, Yamaguchi T, Miyazaki S, Hasegawa M, Wakabayashi H, Asanuma K, Sakaguchi A, Matsumoto T, Ohishi K, Fujimoto N, Yamada N, Ito M, Katayama N, Sudo A: Comparison of three different anti-Xa assays in major orthopedic surgery patients treated with fondaparinux. Int J Hematol 2016 (in press)

2. 学会発表

1) Matsumoto T, Takahashi T, Usui M, Naitoh K, Wada H, Ohishi K, Katayama N, Isaji I: Elevated soluble platelet glycoprotein VI levels in patients after living donor liver transplantation. XXVth ISTH Congress, Tronto, June 20-25, 2015
2) 池尻誠、和田英夫：三重大学における先天性血栓性素因の遺伝子診断、静脈血栓塞栓症の危険因子―先天性血栓性素因と後天性要因、第9回日本血栓止血学会学術標準化委員会シンポジウム、野村コンファレンスプラザ日本橋、2015年2月28日
3) 青田卓実、和田英夫、松本剛史、山下芳樹、片山直之： 日本血栓止血学会DIC診断基準案の検討、新DIC診断基準案と実際の臨床、野村コンファレンスプラザ日本橋、2015年2月28日
4) 和田英夫：日本血栓止血学会DIC診断基準暫定案検討、日本血栓止血学会・日本救急医学会ジョイントシンポジウム

「重症敗血症とDIC－新しいDIC診断基準とアンチトロンビンの役割-」、第37回日本血栓止血学会学術集会、甲府市総合市民会館（甲府市）、2015年5月21日～23日

H．知的財産権の出願・登録状況

（予定を含む。）

1．特許取得

なし

2．実用新案登録

なし

3．その他

特になし

Ⅳ. 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レイアウト

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

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
Kojima M, Ichinose A, Souri M, Osaki T, Kawai H, Amaki J, Numata H, Miyamoto M, Ogiya D, Tsuboi K, Ogawa Y, Ozawa S, Ando K.	Successful bypass surgery for esophageal carcinoma under adequate factor XIII/13 replacement therapy in a case of intractable autoimmune hemorrhaphilia due to anti-Factor XIII/13 antibodies.	Int J Hematol.	in press	in press	2016
Ichinose A, Osaki T, Souri M; Japanese Collaborative Research Group (JCRG) on AH13 (supported by the Japanese Ministry of Health, Labor, and Welfare).	Clinical features of 32 new Japanese cases with autoimmune haemorrha-philia due to anti-factor XIII antibodies.	Haemophilia.	21(5)	653-8.	2015

Kotake T, Souri M, Takada K, Kosugi S, Nakata S, Ichinose A.	Report of a patient with chronic intractable autoimmune hemorrhaphilia due to anti-factor XIII/13 antibodies who died of hemorrhage after sustained clinical remission for 3 years.	Int J Hematol.	101(6):	598-602.	2015
Osaki T, Sugiyama D, Magari Y, Souri M, Ichinose A.	Rapid immunochromatographic test for detection of anti-factor XIII A subunit antibodies can diagnose 90 % of cases with autoimmune haemorrhaphilia XIII/13.	Thromb Haemost.	113(6):	1347-56.	2015
Souri M, Osaki T, Ichinose A.	Anti-factor XIII A subunit (FXIII-A) autoantibodies block FXIII-A_2B_2 assembly and steal FXIII-A from native FXIII-A_2B_2.	J Thromb Haemost.	13(5):	802-14.	2015

Int J Hematol
DOI 10.1007/s12185-015-1917-7

CASE REPORT

Successful bypass surgery for esophageal carcinoma under adequate factor XIII/13 replacement therapy in a case of intractable autoimmune hemorrhaphilia due to anti-Factor XIII/13 antibodies

Minoru Kojima[1] · Akitada Ichinose[2,3] · Masayoshi Souri[2,3] · Tsukasa Osaki[2,3] · Hidetsugu Kawai[1] · Jun Amaki[1] · Hiroki Numata[1] · Mitsuki Miyamoto[1] · Daisuke Ogiya[1] · Kosuke Tsuboi[4] · Yoshiaki Ogawa[1] · Soji Ozawa[5] · Kiyoshi Ando[1]

Received: 15 September 2015 / Revised: 16 November 2015 / Accepted: 18 November 2015

Abstract Autoimmune hemorrhaphilia due to anti-factor XIII (FXIII) antibodies (AH13) is a life-threatening disease associated with high risk of surgical bleeding. Since AH13 occurs mainly in the elderly, patients of AH13 tend to be complicated with other life-threatening diseases that may require surgical procedures. During our nation-wide survey on AH13, supported by the Japanese Ministry of Health, Labor, and Welfare, patients with unexplained bleeding were examined for FXIII-related parameters and anti-FXIII autoantibodies. A 64-year-old man had previously been tentatively diagnosed with AH13 and received immunosuppressive therapies, as FXIII inhibitor was detected by functional cross-mixing studies. About 2 years later, he was definitively diagnosed with AH13, because our immunochromatographic test and enzyme-linked immuno-sorbent assay detected FXIII-bound anti-FXIII-A subunit autoantibodies. Since routine endoscopic examination revealed suspected esophageal carcinoma, a preparatory FXIII pharmacokinetic (PK) analysis was performed by infusing FXIII concentrates prior to biopsy. Consequently, biopsy of this lesion was done without bleeding complications. One month later, a second PK study was carried out before surgery, and esophageal bypass surgery was completed successfully under FXIII replacement therapy. Our experience with this case suggests that operations can be performed safely and with confidence even in patients with such life-threatening hemorrhagic diseases.

Keywords Autoimmune/acquired hemorrhaphilia · Anti-FXIII/13 antibodies · Factor XIII concentrates · Pharmacokinetic study · Immunosuppressive therapy

✉ Akitada Ichinose
aichinos@med.id.yamagata-u.ac.jp

1 Division of Hematology/Oncology, Department of Internal Medicine, Tokai University School of Medicine, Isehara, Japan

2 Department of Molecular Patho-Biochemistry and -Biology, Yamagata University School of Medicine, Yamagata, Japan

3 Japanese Collaborative Research Group on AHXIII/13 (Japanese Ministry of HLW), Yamagata, Japan

4 Department of Internal Medicine, Nagaoka Chuo General Hospital, Nagaoka, Japan

5 Department of Gastroenterological Surgery, Tokai University School of Medicine, Isehara, Japan

Published online: 30 November 2015

Introduction

Factor XIII (FXIII or FXIII/13 to avoid confusion with factor VIII/8 or factor XII/12) is a plasma pro-transglutaminase, which is activated to active transglutaminase, FXIIIa, by thrombin at the final stage of the blood coagulation reaction. FXIII is also called fibrin stabilizing factor because FXIIIa increases the strength of a fibrin clot by cross-linking fibrin monomers to themselves, α_2-plasmin inhibitor (α_2-PI; ISTH Nomenclature in 1972), or fibronectin [1]. Inherited FXIII deficiency is a rare life-long severe bleeding disorder including spontaneous intracranial hemorrhage, and female patients manifest recurrent miscarriage. Some patients also show abnormal wound healing [2].

In the twenty-first century, Japan has become a first super-aging society in the world (population ages 65 and above as a percentage of the total population is 25.1 % in 2013; source, The United Nations Population Division's World Population Prospects). Incidentally, "the number of diagnosed cases" with autoimmune hemorrhagic disease (hemorrhaphilia) due to anti-FXIII autoantibodies (termed AH13) has been on the rise in Japan (8 cases in the last century vs. 47 case in this century; Refs. [3, 4] and unpublished data as of April 2015), mostly in the elderly [3–6]. It is very likely that our nation-wide survey and effort to diagnose AH13 identified more patients than former times [3, 4]. AH13 is a life-threatening hemorrhagic disease [7–9], and some patients are accidentally recognized and diagnosed after they manifest severe postoperative bleedings. Their hemorrhage pattern is also characterized as "delayed bleeding" that occurs 12–36 h after trauma or invasive procedures [1].

On the contrary, if AH13 cases were diagnosed beforehand, operations would be withheld by surgeons, in general, mainly because of the deliberation on excessive/abnormal bleeding. However, even female patients with inherited FXIII deficiency can safely deliver babies under FXIII replacement therapy [10, 11]. This holds true for surgery in cases with inherited FXIII deficiency [12, 13], so why is surgery not performed in AH13 cases? To the authors' best knowledge, there was a single AH13 case for which coronary artery bypass surgery was performed using off-pump, beating heart surgery to minimize risks of a postoperative coagulopathy [14].

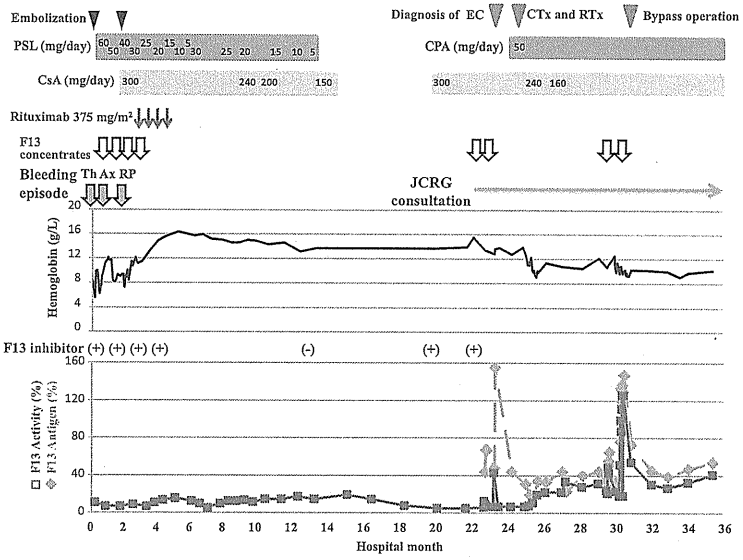

Fig. 1 Clinical course of the present patient. Hemoglobin levels (*black line*) corresponded to bleeding episodes (*arrows with bleeding sites*). Plasma-derived FXIII concentrates were given to arrest bleeding. The patient underwent immune-suppressive therapies first with prednisolone (PSL), then with cyclosporine A (CsA), and finally with rituximab. Although his FXIII activity (*filled squares*) remained low, the FXIII inhibitor (measured by 1:1 cross-mixing test) disappeared 1 year later, and thus CsA treatment was discontinued. Since his FXIII activity returned to 4 % and his FXIII inhibitor re-appeared, CsA treatment was re-started. Then esophageal carcinoma (EC) was suspected by an endoscopic examination. The Japanese collaborative research group (JRCG) was called into consultation and their detailed studies were carried out. He was definitely diagnosed as AH13 by immunological assays, and thus cyclophosphamide (CPA) was added. Under FXIII replacement therapy, biopsy of the esophageal lesion and then its bypass surgery were carried out without hemorrhagic complication, followed by cisplastin treatment (CTx) and radiation therapy (RTx). Both FXIII activity (*filled squares*) and antigen (*filled diamonds*) started to increase gradually. *Ty* thigh, *Ax* axillar, *RP* retroperitoneal hematoma

Case presentation

A previously otherwise healthy 64-year-old Japanese man was hospitalized for a left femur hematoma after a bicycle accident a month earlier (Dec. 201X; Fig. 1, arrow with 'Th'). There was no personal or family history of bleeding diathesis. He underwent embolization of the left femoral artery, because computed tomography (CT; Fig. 2a)

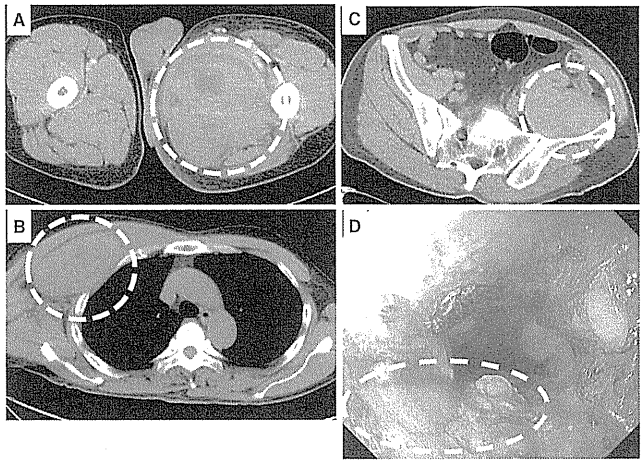

Fig. 2 Computed tomographic images and an endoscopic picture. a Left femoral hematoma at first visit (Jan. 201X + 1). b Right axillar hematoma at first visit (Jan. 201X + 1). c Retro-peritoneal hematoma during clinical exacerbation (Mar. 201X + 1). d Esophageal carcinoma suspected by upper gastrointestinal endoscopy (Dec. 201X + 2). Each lesion is enclosed by a *white broken circle/oval*

and angiography revealed its active bleeding. The patient noticed a hematoma at his right axilla 5 days after embolization (Fig. 1, arrow with 'Ax') and visited our hospital (Jan. 201X + 1; Fig. 2b). His height and body weight were 168 cm and 68 kg.

A physical examination revealed a hematoma of the right axilla as well as a huge hematoma of the left femur accompanied with purpura. Laboratory tests showed low levels of hemoglobin (71 g/L) and red blood cell count (2.3×10^{12}/L), while leukocyte and platelet numbers, C-reactive protein and fibrinogen levels were above the normal ranges (14.2 and 412×10^{9}/L, 81.3 and 426 mg/mL, respectively). D-Dimer (1.1 μg/mL) and fibrin/fibrinogen degradation products (FDP; 4.5 μg/mL), thrombin–antithrombin complex (>60 ng/mL), and von Willebrand factor (VWF; 323 %) activity levels were also increased slightly or moderately, reflecting the patient's severe bleeding condition. Antithrombin and plasmin–plasmin inhibitor complex (PIC) levels were within the normal limits. Routine coagulation screening tests also showed normal prothrombin time and activated partial thromboplastin time. However, his FXIII activity was as low as 10 % (normal range 70–140 %) on Jan. 30, 201X + 1. A cross-mixing study in which the patient's plasma was mixed at a 1:1 ratio with a healthy control's plasma demonstrated significantly low residual FXIII activity (10 %) on Feb. 1, 201X + 1 (FXIII activities of a healthy control and the patient; 108 and 11 %, respectively). He was therefore diagnosed with hemorrhagic acquired FXIII deficiency resulting from FXIII inhibitor, i.e., AH13, tentatively, and thus started to take prednisolone (PSL) at 1 mg/kg, cyclosporine A (CyA) at 5 mg/kg and received plasma-derived FXIII concentrates (Fibrogammin P®, CSL Behring, Tokyo, Japan; 240 U/vial according to the manufacturer's definition). Upper gastrointestinal endoscopic examination revealed no abnormal findings in Jan. 201X + 1, and tumor markers, such as carcino-embryonic antigen, carbohydrate antigen 19-9, and prostate-specific antigen, were not elevated in Jan. 201X + 1.

The patient's bleeding appeared to be temporarily stopped, but his blood pressure and hemoglobin level suddenly dropped a few days later. CT imaging revealed a retroperitoneal hematoma (Fig. 1, arrow with 'RP', Fig. 2c), and thus red cell concentrates (RCC) and FXIII concentrates were transfused, and embolization was performed. To reinforce immunosuppressive therapy, he was also treated with weekly rituximab, four times (Fig. 1). His FXIII activity increased to 15 %, but then decreased to 10 %. After 8 months, FXIII activity increased to 20 % and 1:1 cross-mixing test became negative for FXIII inhibitor, so that CyA was discontinued.

Four months later, FXIII activity decreased again to as low as 4 % and the 1:1 cross-mixing test returned to be

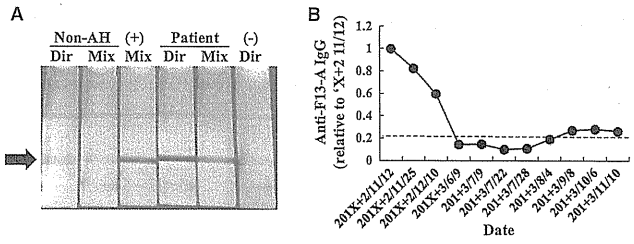

Fig. 3 Japanese collaborative research group (JCRG) analyses of the patient's plasma FXIII and detection of anti-FXIII antibodies (Nov 201X + 2). a ICTs to detect FXIII-A-bound anti-FXIII-A autoantibodies with (spiked) or without (direct) pre-mixing pooled normal plasma. From the *left to the right lanes* direct and spiked ICTs for a case with non-autoimmune hemorrhagic FXIII deficiency (Non-AH), spiked ICT for positive control [(+)], i.e., the plasma obtained from a previously diagnosed AH13 case, direct and spiked ICTs for the present AH13 case (patient), and direct ICT for negative control [(−)], i.e., the plasma obtained from a healthy control. b A fair amount of total anti-FXIII-A immunoglobulin G (IgG) was still clearly detected by the ELISA (*filled circles*). After the immunosuppressive therapy with CPA, his total anti-FXIII-A IgG had significantly decreased. A *dashed line* represents an average level of normal controls. *ICT* immuno-chromatography test, *Dir* direct, *Mix* spiked, *AH13* autoimmune hemorrhagic disease due to anti-FXIII autoantibodies, *ELISA* enzyme-linked immuno-sorbent assay, *CPA* cyclophosphamide

positive for FXIII inhibitor. Accordingly, the patient restarted CyA treatment. Because of the chronic intractable nature of his disease, the Japanese collaborative research group (JCRG; chair; Prof. Ichinose of Yamagata University) was called into consultation. When the patient was examined for the presence of anti-FXIII autoantibodies by an immuno-chromatography test (ICT) [15], anti-FXIII-A autoantibodies were positive (Fig. 3a). Our ELISA method also demonstrated the presence of (total) anti-FXIII-A antibodies (Fig. 3b) [16]. Consequently, he was definitely diagnosed as AH13 and reinforcement of immunosuppressive therapy was planned.

Unfortunately, an upper gastrointestinal endoscopy during a routine health checkup revealed suspected esophageal carcinoma in Dec. 201X + 2, about 2 years after the first endoscopic examination (Fig. 2d). Because of his severe FXIII deficiency, he was began cyclophosphamide (CPA) at 50 mg/day and underwent a pharmacokinetic (PK) analysis by infusing FXIII concentrates at 1200 U in preparation for biopsy (Fig. 4a). Because the recovery rate of the PK study was considerably low (about 25 %), a large dose of FXIII replacement therapy at 60 U/kg (equivalent to 120 % increase) was carried out. As a result, the biopsy was performed successfully without excessive/abnormal bleeding 2 weeks later. The patient was diagnosed as having squamous cell carcinoma of esophagus (UtMt, Type 2, T2, N0, M0, stage II; [17]).

He was treated with chemo-radiotherapy because his severe FXIII deficiency made him ineligible for a curative operation. He received 60 Gy/30 Fr radiotherapy with concurrent chemotherapy (5-FU at 700 mg/m^2 on days 1–4 and cisplatin at 70 mg/m^2; Fig. 1). His esophageal carcinoma went into complete remission and his FXIII activity rose to about 20 %. However, the carcinoma recurred 2 months later. His body weight suddenly decreased to 58 kg due to swallowing disturbance because of esophageal stricture. Accordingly, he required an esophageal bypass operation. In preparation for surgery, an FXIII PK study (dosing 1440 U) was again conducted 3 weeks before (Fig. 4b). Since a good recovery rate of 77 % was obtained, the patient was given 50 U/kg of FXIII concentrate before entering the operating room to achieve a goal of at least 100 % FXIII activity (Fig. 4c). Esophageal bypass and jejunostomy creation were performed. The operation lasted a total of 4 h and 7 min, and the total blood loss during the operation was 302 g, which was the same as that in patients without a bleeding disorder [18], and he received a blood transfusion of 4 units of RCC. There were no problems related to the surgery. FXIII concentrates were also infused during the postoperative period (Fig. 4c), in order to prevent possible "delayed bleeding". Immunosuppressive therapy with CPA alone was continued, whereas CyA was not administered on the day of operation until postoperative day 7.

No massive bleeding was observed after the surgery. No thrombotic events or problematic wound healing occurred. He was released from the hospital on postoperative day 24.

Discussion

Severe hemorrhagic tendency of AH13 patients seems to be quite a burden for surgeons because they have decision-making responsibilities. However, AH13 is a chronic intractable disease mainly in the elderly [7–9], who tend to

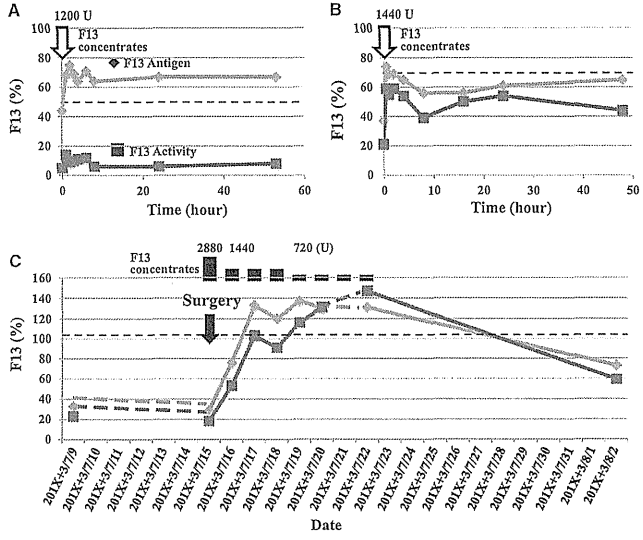

Fig. 4 Preparatory pharmacokinetic (PK) studies (a, b) and hemostatic treatment (c) by infusion of exogenous FXIII. Plasma-derived FXIII concentrates were infused at a dose of 1200 U in Nov. 201X + 2 (17.6 U/kg, equivalent to about 35 % increase) in preparation for biopsy (a) and 1440 U in June 201X + 3 (24.8 U/kg, equivalent to about 50 % increase) in preparation for bypass surgery (b), and during the perioperative period in July 201X + 3 (c). Both FXIII activity (*filled squares*) and antigen levels (*filled diamonds*) were measured before and at the indicated time intervals (a, b) or post-operation dates just before the daily exogenous FXIII infusions (at the *top*; c). *Broken lines* depict calculated FXIII activities after the infusion of exogenous FXIII concentrates. Discrepancies between FXIII activity and antigen levels indicate the formation and existence of FXIII antigen–antibody complexes between "free" anti-FXIII-A autoantibodies and exogenous FXIII concentrates (c). As expected, his FXIII activity reasonably increased from 18 to 53 % on the next day after FXIII concentrates infusion despite he underwent surgical procedure the day before (i.e., in spite of surgical bleeding in addition to the inhibition by anti-FXIII-A autoantibodies and the accelerated clearance of infused FXIII). His FXIII activity kept further increase to 103, 91, 116, 131, and 147 %, because about 30 U/kg FXIII concentrates were administered for the following 3 days and 15 U/kg for another 3 days. We tried to keep his FXIII activity around 100 % of normal at least for several days after surgery in order to prevent the notorious 'delayed bleeding' of severe FXIII deficiency. His FXIII activity then decreased to 59 % 10 days after the discontinuation of FXIII concentrates administration

suffer from other life-threatening diseases, such as cancer, aortic aneurysm, and myocardial infarction, which require surgical procedures. In other words, even if AH13 patients could survive the life-threatening acute stage by proper hemostatic and immunosuppressive treatments, they might be endangered next by aforementioned life-threatening diseases.

Thanks to surgeons, at least two AH13 patients' lives were saved, including a 73-year-old man with AH13 who actually underwent coronary bypass surgery for increasing angina and did well for more than 3 years [14]. Our 64-year-old man with AH13 has also been doing well more than 10 months after esophageal bypass surgery for carcinoma.

It is important to emphasize that malignancies were one of the leading underlying diseases among Japanese AH13 cases [3, 4]. There were 2 cases with gastric cancer, 2 cases with bladder cancer, 1 patient with seminoma, and 1 patient with skin cancer (11 % of all Japanese AH13 cases; Refs. [3, 4] and unpublished data of JCRG). Surgical treatments are indications for most of these malignancies, in general. This is also true in patients with acquired hemophilia A (AHA) caused by auto-antibodies against factor VIII/8, in which malignancies are one of the most common underlying diseases (14.7 % of 150 cases in Ref. [19]). These facts suggest that malignancies may be related to compromised immune reaction and, to some extent, to production of auto-antibodies against these coagulation factors. It was

reported that treatment of cancer with chemotherapy or surgery was followed by the eradication of the inhibitor in 5 out of 41 AHA patients with cancer [20].

We recommended an administration trial of FXIII for the diagnosis of AH13 [6]. It may be also useful to make a hemostatic plan in terms of subsequent dosage and dosing intervals. In fact, PK studies were performed in a 2.5-year-old boy and a 53-year-old man with severe inherited FXIII deficiency before epilepsy neurosurgery [12] and a surgery for aortic valve replacement [13], respectively. Furthermore, it was done in a 73-year-old man with AH13 before coronary artery bypass surgery for angina, although the timing of survival study was not specified [14]. In the present case of AH13, a PK analysis was carried out before esophageal bypass surgery for carcinoma. Surgeries for all four patients were completed successfully, regardless of whether the FXIII deficiency was inherited or acquired, in other words, with or without anti-FXIII autoantibodies. Thus, preparatory PK studies seemed to be very useful to predict the efficacy of FXIII replacement therapy during and after surgery.

There are no evidence-based guidelines on how to manage perioperative therapy for FXIII deficiency, regardless of whether it is inherited or acquired. In particular, few data are available on FXIII dosing perioperatively. In inherited FXIII deficiency, a surgery was performed under FXIII replacement therapy at a dose of 36 U/kg for intracranial surgery [12], and major or minor surgeries were conducted at preoperative doses ranging from 25 to 40 U/kg [13]. Among AH13 cases, a 73-year-old man was given 42 U/kg FXIII for coronary artery bypass surgery [14]. In the present AH13 case, we administered 50 U/kg FXIII concentrates for esophageal bypass surgery, and tried to keep the patient's FXIII level around 100 % of normal for several days after operation.

All these cases demonstrate that major surgeries can be quite safely performed in patients with severe FXIII deficiency, regardless of the presence or the absence of anti-FXIII antibodies.

Acknowledgments This study was approved by the institutional review board of Yamagata University School of Medicine. All procedures were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from this patient. This study was supported in part by research aids from the Japanese Ministry of Health, Labor, and Welfare and a research grant from Yamagata University. We would like to thank all members of 'Japanese Collaborative Research Group (JCRG) on AH13' and Ms. Yuriko Shibue for their cooperation in conducting a nation-wide survey in Japan from 2009 through 2014. The authors also thank Drs. Koji Okamoto, Kento Sakon and Masahito Yano for their invaluable advices on surgical problems of the present case.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest in association with this study.

References

1. Ichinose A. Physiopathology and regulation of factor XIII. Thromb Haemost. 2001;86:57–65.
2. Kohler HP, Ichinose A, Seitz R, Ariens RA, Muszbek L, Factor XIII And Fibrinogen SSC Subcommittee Of The ISTH. Diagnosis and classification of factor XIII deficiencies. J Thromb Haemost. 2011;9:1404–6.
3. Ichinose A, Souri M, Japanese collaborative research group on "Acquired haemorrha-philia due to factor XIII deficiency". As many as 12 cases with haemorrhagic acquired factor XIII deficiency due to its inhibitors were recently found in Japan. Thromb Haemost. 2011;105:925–7.
4. Ichinose A, Osaki T, Souri M, Japanese collaborative research group on "Acquired haemorrha-philia due to factor XIII deficiency". Clinical features of 32 new Japanese cases with autoimmune haemorrha-philia due to anti-factor XIII antibodies. Haemophilia. 2015;21(5):653–8. doi:10.1111/hae.12677.
5. Ichinose A. Hemorrhagic acquired factor XIII (13) deficiency and acquired hemorrhaphilia 13 revisited. Semin Thromb Hemost. 2011;37:382–8.
6. Ichinose A, Japanese Collaborative Research Group (JCRG) on AH13 Hemorrhagic Acquired Coagulopathies. Inhibitors of factor XIII/13 in older patients. Semin Thromb Hemost. 2014;40:704–11.
7. Sugiyama H, Uesugi H, Suzuki S, Tanaka K, Souri M, Ichinose A. Aggressive fatal case of autoimmune hemorrhaphilia resulting from anti-Factor XIII antibodies. Blood Coagul Fibrinolysis. 2013;24:85–9.
8. Boehlen F, Casini A, Chizzolini C, Mansouri B, Kohler HP, Schroeder V, et al. Acquired factor XIII deficiency: a therapeutic challenge. Thromb Haemost. 2013;109:479–87.
9. Kotake T, Souri M, Takada K, Kosugi S, Nakata S, Ichinose A. Report of a patient with chronic intractable autoimmune hemorrhaphilia* due to anti-Factor XIII/13 antibodies who died of hemorrhage after sustained clinical remission† for three years. Int J Hematol. 2015;101:598–602.
10. Ichinose A. Factor XIII is a key molecule at the intersection of coagulation and fibrinolysis as well as inflammation and infection control. Int J Hematol. 2012;95:362–70.
11. Naderi M, Eshghi P, Cohan N, Miri-Moghaddam E, Yaghmaee M, Karimi M. Successful delivery in patients with FXIII deficiency receiving prophylaxis: report of 17 cases in Iran. Haemophilia. 2012;18:773–6.
12. Pernod G, Barro C, Arnutti B, Blanc-Jouvan F, Garrel S, Kahn P, et al. Surgery in severe factor XIII deficiency: report of a case of epilepsy neurosurgery and review. Haemophilia. 2003;9:121–4.
13. Janbain M, Nugent DJ, Powell JS, St-Louis J, Frame VB, Leissinger CA. Use of factor XIII (FXIII) concentrate in patients with congenital FXIII deficiency undergoing surgical procedures. Transfusion. 2015;55:45–50.
14. Lim W, Moffat K, Hayward CP. Prophylactic and perioperative replacement therapy for acquired factor XIII deficiency. J Thromb Haemost. 2004;2:1017–9.
15. Osaki T, Sugiyama D, Magari Y, Souri M, Ichinose A. Rapid immunochromatographic test for detection of anti-factor XIII A subunit antibodies can diagnose 90% of cases with autoimmune hemorrhaphilia XIII/13. Thromb Haemost. 2015;113:1347–56.
16. Souri M, Osaki T, Ichinose A. Anti-factor XIII A subunit (FXIII-A) autoantibodies block FXIII-A2B2 assembly and steal FXIII-A from native FXIII-A2B2. J Thromb Haemost. 2015;13:802–14.
17. Kuwano H, Nishimura Y, Oyama T, Kato H, Kitagawa Y, Kusano M, et al. Guidelines for diagnosis and treatment of carcinoma of the esophagus April 2012 edited by the Japan Esophageal Society. Esophagus. 2015;12:1–30.

18. Kimura M, Ishiguro H, Tanaka T, Takeyama H. Advanced esophageal cancer with tracheobronchial fistula successfully treated by esophageal bypass surgery. Int J Surg Case Rep. 2015;9:115–8.
19. Collins PW, Hirsch S, Baglin TP, Dolan G, Hanley J, Makris M, et al. Acquired hemophilia A in the United Kingdom: a 2-year national surveillance study by the United Kingdom Haemophilia Centre Doctors' Organisation. Blood. 2007;109:1870–7.
20. Sallah S, Wan JY. Inhibitors against factor VIII in patients with cancer. Analysis of 41 patients. Cancer. 2001;91:1067–74.

Haemophilia The Official Journal of the World Federation of Hemophilia, European Association for Haemophilia and Allied Disorders and the Hemostasis & Thrombosis Research Society

Haemophilia (2015), 21, 653–658 DOI: 10.1111/hae.12677

ORIGINAL ARTICLE *Rare bleeding disorders*

Clinical features of 32 new Japanese cases with autoimmune haemorrha-philia[1] due to anti-factor XIII antibodies

A. ICHINOSE, T. OSAKI and M. SOURI THE JAPANESE COLLABORATIVE RESEARCH GROUP (JCRG) ON AH13 (SUPPORTED BY THE JAPANESE MINISTRY OF HEALTH, LABOR, AND WELFARE)[2]

Department of Molecular Patho-Biochemistry and Patho-Biology, Yamagata University School of Medicine, Yamagata, Japan

Introduction: Autoimmune haemophilia-like disease (or haemorrha-philia) due to anti-factor XIII (FXIII; F13 to avoid confusion with FVIII or FXII) antibodies (termed AH13) is a severe bleeding disorder. Although AH13 is thought to be rare, 'the number of its diagnosed patients' has recently increased in Japan. However, its prevalence remains unknown. **Aim:** To improve understanding of this disease, we examined and diagnosed 32 'new' Japanese patients with AH13. **Methods:** The presence of antibodies against F13-A subunit and/or F13-B subunit was confirmed by using a dot blot test and enzyme-linked immunosorbent assays. **Results:** Most of our patients had autoantibodies against the F13-A subunit (88%). A predominance of men (59%) was observed. The mean age and residual F13 activity of our AH13 cohort were 71.7 years and 10.5% of normal, respectively, and 53% of cases were idiopathic. Autoimmune disorders and malignancies were the leading underlying disease (both 16%). Intramuscular and subcutaneous bleeding were the leading symptoms (both 72%). Most of our patients were treated with F13 concentrates (72%) to arrest bleeding and with prednisolone (81%) to eradicate anti-F13 autoantibodies. Cyclophosphamide and rituximab (both 2.5%) were also administered. The mortality of AH13 was high (22%), and haemorrhage was the major cause of death (71%). Moreover, 13% of our AH13 patients were diagnosed after haemorrhagic death. **Conclusion:** Physicians/haematologists must raise the awareness of AH13 as a life-threatening disease. This report represents the only experience of a nationwide survey, and may contribute to a diagnosis on potentially overlooked non-Japanese AH13 patients in other countries in the world.

Keywords: autoimmune disease, chronic intractable disease, haemostatic therapy, immunosuppressive treatment, life-threatening bleeding disorder, nationwide survey

Introduction

Coagulation factor XIII (FXIII, or F13 is used to avoid confusion with FVIII and FXII for medical safety measure) is a fibrin-stabilizing factor and comprises a hetero-tetramer formed by two catalytic A subunits (F13-A) and two non-catalytic carrier B subunits (F13-B) [1–4]. Congenital F13 deficiency can cause lifelong severe bleeding and abnormal wound healing, and recurrent miscarriages in women. It is a very rare form of haemorrhagic disorder that occurs in only one in 3 million individuals [1], and approximately 600 cases have been identified worldwide [5]. By contrast, acquired F13 deficiency is much more common, frequently characterized by a non-autoimmune secondary decrease in F13 resulting from hypo-synthesis and/or hyper-consumption of F13 due to primary diseases [3,4,6].

'Acquired haemophilia (AH) is an autoimmune disease characterized by the presence of autoantibodies directed against various clotting factors [7]'. The incidence of 'acquired haemophilia A (AHA) due to anti-FVIII(8) inhibitors' has been estimated at 1.5 cases per 1 million population per year [8].

Correspondence: Akitada Ichinose, MD, PhD, Department of Molecular Patho-Biochemistry and Patho-Biology, Yamagata University School of Medicine, Yamagata 990-9585, Japan.
Tel.: +81 23 628 5276; fax: +81 23 628 5280;
e-mail: aichinos@med.id.yamagata-u.ac.jp

[1]Haemophilia-like disease (KM Brinkhous. A short history of haemophilia, with some comments on the word 'Haemophilia'. In: Brinkhous KM, Hemker HC eds. Handbook of Hemophilia, Part 1. Amsterdam, New York: Excerpta Medica, American Elsevier Pub. Co., 1975: 3–20).

[2]The members of Japanese Collaborative Research Group (JCRG) on AH13 are listed in Appendix.

Accepted after revision 13 February 2015

In contrast, data on a few cases of 'autoimmune haemophilia-like disease (or hemorrha-philia) due to anti-F13 antibodies (termed AH13)' have been reported [3,9]. Only 19 cases with anti-F13 autoantibodies or acquired F13 inhibitors (excluding one patient with anti-fibrinogen antibodies and two patients with anti-F13 alloantibodies) were reported in the last century [10]. AH13 must be distinguished from non-autoimmune haemorrhagic acquired F13 deficiency (HAF13-def) [9] in that AH13 is more severe than HAF13def, and requires immunosuppressive therapy to eradicate autoantibodies, as well as F13 replacement therapy to stop bleeding. Nevertheless, even severe F13 deficiency in AH13 patients tends to be overlooked by clinicians, because thus far, there is no routine standard screening test to detect abnormal F13 activity.

The number of diagnosed AH13 cases recently seems to be on the increase in Japan [11] when compared to that of previously reported cases in the last century [12–19]. Accordingly, we investigated exclusively bleeding patients to clarify the actual status of AH13. As a result, we diagnosed 32 new Japanese patients with AH13 in the last 4 years. The 12 patients previously reported in ref. [11] were not included in duplicate in this cohort. Thus, this report represents the only as well as the largest experience of a nationwide survey documented, to date. The present manuscript aimed to characterize the presenting symptoms and outcomes of these AH13 patients to improve understanding of this disease.

Methods and materials

Recombinant F13-A (rF13-A) was a kind gift from Dr. P. Bishop of Zymogenetics (Seattle, WA). rF13-B was expressed using a baculovirus expression system and purified as previously described [20]. An anti-F13-A monoclonal antibody (mAb) was generously provided by Dr. G. Reed of Massachusetts General Hospital (Harvard Medical School, Boston, MA). A tetramethylbenzidine (TMB) peroxidase substrate kit was purchased from Bio-Rad Laboratories (Hercules, CA, USA).

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. Written informed consent was obtained from all participants including AH13 cases of the present study.

All research procedures [inclusion criteria, blood collection, plasma F13 activity, enzyme-linked immunosorbent assay (ELISA) to detect anti-F13-B autoantibodies, dot blot assay for anti-F13-A or anti-F13-B autoantibodies, etc.] have been described previously [11,21,22], except for the ELISA to detect anti-F13-A autoantibodies, as described later. Briefly, since 2009 we officially embarked on a nationwide campaign against AH13, supported by the Japanese Ministry of Health, Labor, and Welfare. Flyers and a simple questionnaire on past cases of various types of acquired haemorrha-philia were sent to approximately 2000 university or public hospitals and haematologists. Patients who were bleeding actively due to unknown causes were recruited into the survey and were examined in detail. Inclusion criteria were [9,11]: healthy individuals who suddenly manifested severe bleeding symptoms without a family history of bleeding disorders, prolonged clotting times or platelet abnormalities, in which cases their physicians contacted members of the study group (Appendix).

Whole blood was collected into tubes containing a one-tenth volume of 3.2% sodium citrate. Plasma samples were quick-frozen and sent to a commercial service laboratory (SRL Ltd., Hachioji, Japan) for measurement of the plasma F13 activity with an ammonia release assay using a Berichrom FXIII kit (Siemens/Sysmex, Kobe, Japan), with a reference range of 70–140%.

Furthermore, suspected cases of AH13 were examined in Yamagata University for the presence of antibodies against F13-A and F13-B by using a dot blot test, as previously described [22]. For an anti-F13-A ELISA, 1 μL of plasma was incubated with 200 ng of rF13-A at 37°C for 2 h, and diluted 100-fold with a buffer. Ten microlitres of the diluted plasma was applied to a 96-well plate coated with anti-F13-A mAb and incubated. The plate was incubated with peroxidase-conjugated anti-human IgG. A 10-min assay was performed using TMB substrate. Cases with HAF13def secondary to other diseases, such as disseminated intravascular coagulation, were excluded easily because of the absence of anti-F13 antibodies.

For statistical analysis, values were expressed as mean ± SD or as median when specified. Comparisons between groups were performed using Kruskal–Wallis tests unless specified, or Fisher's exact tests (2×2 table) when appropriate, of SAS Enterprise Guide 6.1 (SAS Institute, Cary, NC, USA). Differences were significant at a P-value of <0.05.

Results and discussion

Number of diagnosed AH13 cases in Japan

We diagnosed a total of 32 new AH13 cases during the last 4 years, from late 2010 through 2014 (Table 1). During the preceding 7 years, until early 2010, when we consulted physicians in charge of the AH13 cases, there was only 1 or 2 AH13 case per year [11]. However, the number of diagnosed AH13 cases has increased from late 2010, and 15 AH13 cases were diagnosed during 12 months (the Japanese business year between April 2013 and March 2014).

Table 1. Presenting characteristics of two Japanese patients with AH13.

	No.	(%)
Reported/diagnosed year		
2010	2	6
2011	3	9
2012	8	25
2013	12	38
2014	7	22
Total	32	100
Gender		
Female	13	41
Male	19	59
Total	32	100
Age category		
<40	0	0
<60	2	6
<80	23	72
≥80	7	22
Total	32	100
Sites of bleeding		
IM	23	72
SC	23	72
IC	4	13
IP	4	13
GI	3	9
Kidney/urinary	3	9
RetroP	2	6
PostOpe.	2	6
Comp	2	6
IJ	2	6
Spl Rap	1	3
Total	32	
Residual F13 activity		
0 to <5%	7	22
5 to <10%	15	47
10 to <20%	6	19
≥20%	4	13
Low	0	0
Total	32	100
Target of antibody		
IgG to F13-A	28	88
IgG to F13-B	5	16
Anti-F13	2	6
n.d.	0	0
Total	32	
Underlying disease		
None	17	53
Autoimmune disease	6*	19
Cancer	5	16
Diabetes mellitus	4	13
Hepatitis	3†	9
Schizophrenia	2	6
Syphilis	1	3
CML	1	3
Total	32	

n.d., not described; SC, subcutaneous; IM, intramuscular; GI, gastrointestinal; IC, intracranial; IJ, intrajoint; IP, intraperitoneal; PostOpe., operation site; RetroP, retroperitoneal; Comp., compartment syndrome; Spl Rap, spleen rupture; CML, chronic myelogenous leukaemia.
*One patient had two autoimmune diseases.
†Two patients had viral hepatitis.

There were only eight AH13 cases reported in the last century between 1986 and 1997 [12–19].

Because we began a nationwide survey in Japan in April 2009, the awareness of AH13 may have increased. Actually, leaflets, questionnaires and/or annual research report booklets on AH13 have been sent to approximately 2000 hospitals, twice every year since late 2009. In addition, Japan has become a rapidly ageing society, ranking first in the world, and therefore, the Japanese may be more susceptible to AH13, for some unknown reason related to compromised immune-tolerance. Finally, the number of diagnosed AH13 cases may have increased in the 21st century because its diagnosis has evolved over a long period since the last century. It is very likely that a concerted effort to identify these patients identified more patients.

Age and gender

The incidence of AH13 increased with age in our study sample (Table 1). The mean age was 71.7 ± 9.3 years (n = 32, median 70.5 years). There were more patients in the age group 60 to <80 years than other age groups (72%), when compared to patients in other age groups (P = 0.0005). In our cohort, AH13 did not occur in childhood or youth during the last 6-year period.

A predominance of male subjects was observed among our 32 AH13 patients (59% men and 41% women, Table 1), which is similar to the previous cohort [11].

Bleeding sites and symptoms

Bleeding symptoms among our 32 AH13 patients varied considerably (Table 1). Most of them bled in soft tissues, and intramuscular (72%) and subcutaneous (72%) bleeding were the leading sites of haemorrhage. In contrast, postsurgical bleeding was rarely observed (6%).

It is important to observe that intracranial bleeding occurred in 4 of our 32 AH13 cases (13%, Table 1). Furthermore, six patients manifested intraperitoneal or retroperitoneal bleeding (19%). One of our AH13 cases manifested spleen rapture spontaneously. These results indicate that AH13 is one of the most severe life-threatening haemorrhagic diseases.

It is important to note that the bleeding symptoms of AH13 are indistinguishable from those of 149 AHA cases in the United Kingdom [8]. Therefore, AH13 must be differentially diagnosed according to specific coagulation tests as proposed by our collaborative research group [11], but not depending upon haemorrhagic symptoms *per se*.

Residual F13 activity

The mean residual F13 activity was 10.5 ± 8.5% (n = 32; median 7%) among our AH13 patients (Table 1). More patients (15/32 = 47%) exhibited a residual F13 activity between 5% and <10% (arbitrary category) when compared to those with <5% (arbitrary category) of F13 activity (7/32 = 22%). Only one case of the former group had received F13 concentrates before blood collection. There was no clear relationship between the residual F13 activity and bleeding severity, because even patients having >10% of normal F13 activity (9/10, 90%) manifested spontaneous bleeding (Table 1), except for one patient who developed only postoperation bleeding.

These findings may merely reflect the fact that residual F13 activities in patients were measured in a commercial service laboratory using an ammonia release assay, which usually yields high background/blank values [23].

Major targets of F13 autoantibodies

Among our 32 AH13 patients, 88% developed anti-F13-A autoantibodies, whereas only five patients (16%) developed anti-F13-B autoantibodies (P < 0.0001; Table 1). Among them, two patients developed anti-F13-B autoantibodies alone, and the remaining three patients had both anti-F13-A and anti-F13-B autoantibodies.

These results suggest that AH13 patients with anti-F13-B autoantibodies may be overlooked because they usually manifest milder bleeding symptoms, as observed in congenital F13-B deficiency [3,4]. This is consistent with the fact that these two patients had relatively higher residual F13 activities, corresponding to 41%, and 13%, respectively. An exception was a Hungarian patient with severe disease who showed essentially no F13 activity (<1% of the normal values) [24]. Incidentally, F13 activity and F13-B antigen levels are normally not measured by general physicians.

Physicians should understand that immunological assays exhibit greater accuracy and sensitivity [25], and can detect both neutralizing and non-neutralizing antibodies, whereas functional assays detect immediately apparent inhibitors but do not detect non-neutralizing antibodies.

Underlying diseases

No underlying condition was found in 53% of our AH13 patients (Table 1), like AHA patients [8]. Of note, five patients (16%) had malignancies, two patients (6%) presented with viral hepatitis, and one patient presented with syphilis. Incidentally, no AH13 case was associated with pregnancy, in contrast to AHA cases in the United Kingdom [8]. Five AH13 patients had autoimmune disorders, which was also the leading associated disease (16%; Table 1). It is widely accepted that malignancies, pregnancy, and infectious and autoimmune diseases are related to compromised immune reaction to some extent.

Haemostatic treatment

Because of the presence of severe F13 deficiency due to anti-F13 autoantibodies, F13 concentrates were administered to most of our AH13 patients (72%, Table 2). Fortunately, F13 concentrates were approved by the public medical services tentatively in April 2013 and officially in September 2013 in Japan through our efforts.

Fresh frozen plasma (FFP) was less frequently infused into our patients (22%), while cryoprecipitate was not administered at all (cryoprecipitate is not commercially available in Japan). In addition, recombinant activated FVII (FVIIa) was not used in our AH13 cases [unpublished data of Japanese Collaborative Research Group (JCRG)], probably because AH13 patients have normal amounts of endogenous FVII, and consequently, do not need exogenous FVIIa. Unfortunately, there is neither an alternative nor bypassing agents for F13. This is a major difference from the haemostatic therapy for AHA [25,26].

Anti-fibrinolytic agents, such as tranexamic acid, were used in 44% of our AH13 patients (Table 2). Twelve of 16 physicians (75%) observed favourable effects of tranexamic acid on haemostasis in their patients (unpublished data of JCRG).

Table 2. Management and prognosis of AH13 cases.

	No.	(%)
Haemostatic treatment		
F13	23	72
RCC	6	19
FFP	7	22
Tr	14	44
Ad	3	9
None	3	9
n.d./unknown	1	3
Total	32	
Ab eradication		
PSL	26	81
CP	8	25
RTX	8	25
CS	4	13
Pulse	4	13
PE	2	6
IVIg	1	3
None	3	9
n.d./unknown	1	3
PSL only	9	28
Total	32	
Prognosis/outcome		
Recovered	5	16
Death	7	22
Under treatment	18	56
Monitoring	2	6
Total	32	100

n.d., not described; Ab, antibody; Ad, adrenalin; FFP, fresh frozen plasma; IVIg, intravenous immunoglobulin; RCC, red cell concentrates; Tr, tranexamic acid; CP, cyclophosphamide; CS, cyclosporine; PE, plasma exchange; PSL, predonisolone; Pulse, steroid pulse; RTX, retuximab.

Antibody eradication therapy

Most of our AH13 patients (81%) were initially treated with prednisolone (PSL; Table 2). Cyclophosphamide was administered as second-line therapy (25% of all cases). Steroid therapy alone may not be sufficient to suppress autoantibody production because only one out of our nine AH13 patients who were treated with PSL alone recovered (data not shown).

Rituximab, an anti-CD20 mAb, has been used in 25% AH13 patients (Table 2). Although RTX has been used in Japanese AH13 patients since 2010, it still has not been approved for AH13 treatment by the public medical insurance in Japan, and its off-label use is restricted for Japanese physicians who treat AH13 cases.

There was a scarcity in the use of high-dose intravenous immunoglobulin (3%) and the performance of plasma exchange (6%) in our AH13 patients. High-dose intravenous immunoglobulin is not recommended for AHA patients [26]. Of note, the effect of plasma exchange and immune-adsorption is transient because these procedures only remove or reduce anti-F13 autoantibodies in patents' plasma, but do not interrupt the synthesis of these autoantibodies.

The optimal immunosuppressive strategy has not been defined for AH13 yet. As more data become available by accumulating experience, the efficacy of these treatment regimens may justify recommendations of novel first-line therapies for AH13.

Outcome and prognosis

Unfortunately, only 16% of our patients primarily recovered from AH13, and 56% continued receiving treatment (Table 2). Moreover, seven patients (22%) died within a year. Five of the seven deaths (71%) were due to or related to haemorrhage (Table 3). Therefore, AH13 is still a life-threatening haemorrhagic disease. Moreover, among our 32 patients, four were 'dead on arrival of test samples'. These findings emphasize the need to raise the awareness of this disease.

Two AH13 patients have been on treatment for more than 2.5 years, even though they had successfully survived the life-threatening acute phase, which reinforces that AH13 should be considered a chronic intractable disorder.

The mean age of the seven non-survived patients (66.7 ± 12.0 years, Table 3) was lower than that of survived AH13 patients (73.0 ± 8.2 years, $n = 25$, $P = 0.12$; *ns*). No significant differences in the mean residual F13 activity were observed between non-survived patients (5.6 ± 1.6%) and survived cases (11.9 ± 10.8%, $P = 0.35$).

Effect of treatment on survival

Four of our seven (57%) patients who had received FFP died (vs. 3 out of 25 patients without FFP, 12%, $P = 0.026$ by Fisher's exact test). Two of seven non-survived patients (29%) did not receive F13 concentrates.

A third of our patients (3 out of 9 patients, 33%) who received only steroids died (vs. 7 deaths out of all our 32 patients, 22%, $P = 0.66$ by Fisher's exact test). By contrast, all four patients (100%, $P = 0.55$ by Fisher's exact test) who had undergone pulse steroid therapy survived.

Unfortunately, at least one of our 32 AH13 patients may have died of pneumonia during steroid therapy, and another patient has died from intestinal perforation during steroid tapering (Table 3). These results highlight the need to seriously consider the adverse effects of immunosuppressive therapies, as recommended for the treatment of AHA [8].

Conclusion

This manuscript represents the one and only as well as the largest and longest duration experience of a nationwide survey reported, to date. AH13 is a chronic intractable disease. Affected patients require long-term medical care. At the same time, AH13 is definitely a life-threatening disease. Unless treated promptly and aggressively [27], patient may die of haemorrhage either during the acute stage [28] or after a prolonged period. Finally, the prompt diagnosis and treatment of AH13 are essential to save patients' lives. We strongly recommend a long-term follow-up

Table 3. Cause and timing of death of AH13 cases.

Age	F13 activity (%)	Haemostatic treatment	Cause of death	Timing of death	
66	6	RCC, FFP	Bleeding (no F13 dosing)	1 week before DOAS	7 weeks after admission
65	6	F13	Cerebral bleeding	1 week before DOAS	3 weeks after admission
60	7	F13, RCC, FFP, Tr	Haemorrhagic shock	5 weeks before DOAS	3 weeks after admission
79	7	Tr	Cerebral bleeding (no F13 dosing)	4 months before DOAS	2.5 months after admission
63	20*	F13, RCC, FFP, Tr, Ad	Suicide because of bleeding	4.5 months after diagnosis	6 months after admission
49	4	F13, FFP, Tr	Intestinal perforation	11 months after diagnosis	During steroid tapering
85	3	F13	Sepsis from infected skin ulcer	1 year after diagnosis	9 months after eradication off

Ad, adrenalin; F13 conc., F13 concentrates; FFP, fresh frozen plasma; RCC, red cell concentrates; Tr, tranexamic acid; DOAS, dead on arrival of test plasma samples to the first author's laboratory.
*After F13 conc. infusion.

of AH13 patients, for several years following remission, similar to the strategy used for AHA [26]. If clinical symptoms indicate a relapse, testing and treatment should be initiated immediately.

Acknowledgements

This study was supported by research grants from the Japanese Ministry of Health, Labor, and Welfare for 5 years. We are deeply indebted to over 150 physicians for consulting their patients with severe acquired haemorrha-philia including AH13, Acquired von Willebrand syndrome, AHA, etc. We wish to thank Drs. Magari Y and Sugiyama D of Q-may Laboratory Inc. for their help in providing an immuno-chromatographic test kit to rapidly detect anti-F13-A autoantibodies and Ms. Shibue Y for her help in conducting this research project and in the preparation of this manuscript.

Authorship contribution:

AI planed and conducted the project, analysed data and wrote the paper. TO and MS carried out experimental examinations, analysed data and prepared the paper.

Disclosures

The authors stated that they had no interests which might be perceived as posing a conflict or bias.

References

1 Nugent DJ. Prophylaxis in rare coagulation disorders – factor XIII deficiency. *Thromb Res* 2006; 11851: S23–8.
2 Kohler HP, Ichinose A, Seitz R *et al.* Diagnosis and classification of factor XIII deficiencies. *J Thromb Haemost* 2011; 9: 1404–6.
3 Ichinose A. Factor XIII is a key molecule at the intersection of coagulation and fibrinolysis as well as inflammation and infection control. *Int J Hematol* 2012; 95: 362–70.
4 Biswas A, Ivaskevicius V, Thomas A *et al.* Coagulation factor XIII deficiency. Diagnosis, prevalence and management of inherited and acquired forms. *Hamostaseologie* 2014; 34: 160–6.
5 Shetty S, Shelar T, Mirgal D *et al.* Rare coagulation factor deficiencies: a country-wide screening data from India. *Haemophilia* 2014; 20: 575–81.
6 Egbring R, Kröniger A, Seitz R. Factor XIII deficiency: pathogenic mechanisms and clinical significance. *Semin Thromb Hemost* 1996; 22: 419–25.
7 Boggio LN, Green D. Acquired hemophilia. *Rev Clin Exp Hematol* 2001; 5: 389–404.
8 Collins PW, Hirsch S, Baglin TP *et al.* Acquired hemophilia A in the United Kingdom: a 2-year national surveillance study by the United Kingdom Haemophilia Centre Doctors' Organisation. *Blood* 2007; 109: 1870–7.
9 Ichinose A. Hemorrhagic acquired factor XIII (13) deficiency and acquired hemorrhaphilia 13 revisited. *Semin Thromb Hemost* 2011; 37: 382–8.
10 Lorand L. Acquired inhibitors of fibrin stabilization: a class of hemorrhagic disorders of diverse origins. In: Green D ed. Anticoagulants, Physiologic, Pathologic and Pharmacologic. Boca Raton, FL: CRC Press, 1994: 169–91.
11 Ichinose A, Souri M. Japanese Collaborative Research Group on 'Acquired haemorrha-philia due to factor XIII deficiency'. As many as 12 cases with haemorrhagic acquired factor XIII deficiency due to its inhibitors were recently found in Japan. *Thromb Haemost* 2011; 105: 925–7.
12 Teruya J, Kinoshita T, Yoshimura Y *et al.* An inhibitor to factor VIII interacting with its activated catalytic subunit. *Rinsho Ketsueki* 1986; 27: 1680–7, Japanese.
13 Nakamura S, Kato A, Sakata Y *et al.* Bleeding tendency caused by IgG inhibitor to factor XIII, treated successfully by cyclophosphamide. *Br J Haematol* 1988; 68: 313–9.
14 Ino H, Shiomi Z, Hayami H *et al.* A case suspected of acquired factor XIII abnormality [abstract]. *Int J Hematol* 1984; 47: 650, Japanese.
15 Kato A, Saito T, Kiyose H *et al.* Successful steroid therapy of acquired coagulation factor XIII insufficiency [abstract]. *Rinsho Ketsueki* 1988; 29: 908, Japanese.
16 Nakazawa M, Iida R, Tanoue K *et al.* Successful gastrectomy in a patient with inhibitor activities to factor XIII and phospholipid-mediated clotting time [abstract]. *Thromb Haemost* 1997; 35: PS-137.
17 Shinkai K, Nishiwaki H, Takagi A *et al.* Hemostatic management of severe hemorrhage in a case with factor XIII inhibitor [abstract]. *Int J Hematol* 1996; 63(Suppl. 1): 291, Japanese.
18 Kohro J, Nakamura K, Yamamoto T *et al.* A case of SLE complicated by coagulation factor XIII deficiency [abstract]. *Rynmachi* 1997; 37: 326, Japanese.
19 Kanto H, Kurikawa Y, Koseki M *et al.* A case of annular elastolytic giant cell Granuloma Associated with acquired plasma factor XIII deficiency. *Jpn J Dermatol* 1998; 108: 747–54, Japanese.
20 Souri M, Koseki-Kuno S, Takeda N *et al.* Administration of factor XIII B subunit increased plasma factor XIII A subunit levels in factor XIII B subunit knock-out mice. *Int J Hematol* 2008; 87: 60–8.
21 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* 2012; 95: 47–50.
22 Wada H, Souri M, Matsumoto R *et al.* Alloantibodies against the B subunit of plasma factor XIII developed in its congenital deficiency. *Thromb Haemost* 2013; 109: 661–8.
23 Ajzner E, Muszbek L. Kinetic spectrophotometric factor XIII activity assays: the subtraction of plasma blank is not omissible [corrected]. *J Thromb Haemost* 2004; 2: 2075–7.
24 Ajzner E, Schlammadinger A, Kerényi A *et al.* Severe bleeding complications caused by an autoantibody against the B subunit of plasma factor XIII: a novel form of acquired factor XIII deficiency. *Blood* 2009; 113: 723–5.
25 Green D. Factor VIII inhibitors: a 50-year perspective. *Haemophilia* 2011; 17: 831–8.
26 Huth-Kühne A, Baudo F, Collins P *et al.* International recommendations on the diagnosis and treatment of patients with acquired hemophilia A. *Haematologica* 2009; 94: 566–75.
27 Ogawa Y, Mihara M, Souri M *et al.* Complete remission achieved by steroid pulse therapy following rituximab treatment in a case with autoimmune haemorrhaphilia due to anti-factor XIII antibodies. *Thromb Haemost* 2014; 112: 831–3.
28 Sugiyama H, Uesugi H, Suzuki S, Tanaka K, Souri M, Ichinose A. Aggressive fatal case of autoimmune hemorrhaphilia resulting from anti-factor XIII antibodies. *Blood Coagul Fibrinolysis* 2013; 24: 85–9.

Appendix

Following were members of 'the Japanese Collaborative Research Group (JCRG) on AH13' for cooperation in conducting the project; Ichinose A, Souri M, Osaki T, Matsushita T, Urano T, Ieko M, Tamai Y, Ito T, Kawamae K, Yamamoto M, Madoiwa S, Kurosawa H, Ogawa Y, Yatomi Y, Hanafusa N, Kawasugi K, Ishida F, Kitajima I, Asakura H, Hosono N, Mouri H, Kobayashi T, Wada H, Miyata S, Kashiwagi H, Ikeda M, Yukawa M, Higasa S, Hato T, Okamoto K, Okamura T, Uchiba M, Hashiguchi T and Maruyama I.

Int J Hematol (2015) 101:598–602
DOI 10.1007/s12185-015-1754-8

CASE REPORT

Report of a patient with chronic intractable autoimmune hemorrhaphilia due to anti-factor XIII/13 antibodies who died of hemorrhage after sustained clinical remission for 3 years

Takeshi Kotake · Masayoshi Souri · Koji Takada · Satoru Kosugi · Soichi Nakata · Akitada Ichinose

Received: 16 December 2014 / Revised: 22 January 2015 / Accepted: 26 January 2015 / Published online: 8 February 2015

Abstract Although the incidence of autoimmune hemorrhaphilia due to anti-Factor XIII (FXIII, not FVIII or FXII to avoid confusion) antibodies (AH13) or hemorrhagic "acquired FXIII deficiency due to anti-FXIII autoantibodies" was previously considered rare, it has been on the increase in the twenty-first century, at least in Japan. An 83-year-old woman with an unexplained hemorrhage was admitted to our hospital for intramuscular hematoma and severe anemia. Her FXIII activity was reduced to 10 % of normal; since FXIII inhibitors and anti-FXIII-A subunit autoantibodies were detected, she was definitively diagnosed with AH13. Despite developing cardiac tamponade due to pericardial hemorrhage, she clinically recovered from AH13 after hemostatic therapy with FXIII-concentrates and immunosuppressive treatment with rituximab and cyclophosphamide. However, her FXIII activity remained low and she died of hemorrhage 3.5 years after admission. AH13 patients should be monitored for a prolonged period, as this disease is very likely a chronic intractable hemorrhagic disorder.

Keywords Autoimmune disease · Hemorrhagic disorder · Chronic disease · Hemostatic therapy · Immunosuppressive treatment

Acquired h(a)emophilia is a tentative name for this category of diseases, but unofficial because it is not included in the current version of the WHO ICD-10. "Acquired h(a)emorrhaphilia" seems to be a more logical and proper appellation, because the term hemorrhaphilia stands for "love of bleeding/hemorrhage" while the word hemophilia literally means "love of blood" [Brinkhous, K.M. A short history of hemophilia, with some comments on the word "Hemophilia". In: KM Brinkhous and HC Hemker (eds). Handbook of Hemophilia, part 1, Amsterdam: Excerpta Medica, American Elsevier. 1975, p. 3–20]. Thus, the authors use the term hemorrhaphilia for a bleeding disorder caused by anti-FXIII autoantibodies, throughout this manuscript. H(a)emophilia must be used for the inherited hemorrhagic disease due to Factor VIII deficiency as listed in WHO ICD-10.

'Clinical' remission is defined as the disappearance of all bleeding symptoms, in this manuscript.

Masayoshi Souri and Akitada Ichinose belong to Japanese Collaborative Research Group on AH13.

Electronic supplementary material The online version of this article (doi:10.1007/s12185-015-1754-8) contains supplementary material, which is available to authorized users.

T. Kotake · S. Kosugi · S. Nakata
Department of Internal Medicine,
Toyonaka Municipal Hospital, Toyonaka,
Osaka, Japan

Present Address:
T. Kotake
Department of Medical Oncology, Kobe City Medical Center
General Hospital, Kobe, Hyogo, Japan

M. Souri · A. Ichinose (✉)
Department of Molecular Patho-Biochemistry and Patho-Biology,
Yamagata University School of Medicine, Yamagata 990-2331,
Japan
e-mail: aichinos@med.id.yamagata-u.ac.jp

K. Takada
Department of Anesthesia and Intensive Care, Toyonaka
Municipal Hospital, Toyonaka, Osaka, Japan

Introduction

Factor XIII (FXIII not FVIII or FXII) is a fibrin-stabilizing factor in the plasma consisting of A and B subunits (FXIII-A and FXIII-B, respectively). Because thrombin-activated FXIII cross-links fibrin monomers themselves and fibrin and α_2-plasmin inhibitor, congenital FXIII deficiency results in a lifelong severe bleeding tendency [1, 2]. Although congenital FXIII deficiency is rare [3], acquired FXIII deficiency is rather common, and mostly secondary to hyper-consumption or hypo-synthesis of FXIII (disseminated intravascular coagulation, surgery, trauma, leukemia, liver diseases, chronic inflammatory bowel diseases, artificial dialysis, etc.) [4]. However, patients with acquired FXIII deficiency rarely bleed because their FXIII levels remain only moderately reduced [5, 6]. In contrast, patients with autoimmune hemophilia-like disease (i.e., hemorrhaphilia) due to anti-FXIII antibodies (AH13) or hemorrhagic "acquired FXIII deficiency due to anti-FXIII autoantibodies" experience severe bleeding due to drastically decreased FXIII levels and the consequently reduced cross-linked α_2-plasmin inhibitor to fibrin [7].

Although AH13 used to be rare [8, 9], its incidence has been on the rise in the twenty-first century in Japan [10]. It is very likely because Japan has become a super-aging society first in the world, and because we have conducted a nation-wide survey supported by the Japanese Ministry of Health, Labor and Welfare. In this study, we report an 83-year-old woman with chronic intractable AH13.

Methods

This study was approved by the institutional review board of Yamagata University School of Medicine. All procedures were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from this patient.

All experimental procedures have been described previously [11, 12] except for the enzyme-linked immunosorbent assay (ELISA) to detect anti-FXIII-A autoantibodies: briefly, 1 μL of plasma was incubated with 200 ng of recombinant (r)FXIII-A in a 10 μL mixture at 37 °C for 2 h and then diluted tenfold with a buffer. Ten microliters of the diluted plasma was added to a 96-well plate coated with anti-FXIII-A monoclonal antibody and reaction with peroxidase-conjugated anti-human Immunoglobulin G (IgG) was allowed for 60 min. ELISA was also carried out without adding rFXIII-A into patient's plasma to detect pre-existing anti-FXIII-A autoantibodies bound to own FXIII-A.

For dot blot assay [11], 50 and 100 ng of either rFXIII-A or rFXIII-B, or 100 and 200 ng of rFXIII complex (A_2B_2 tetramer) were blotted onto a nitrocellulose membrane, and reacted with the patient's plasma at a dilution of 1:2,000. Immunoglobulin bound to either one of these FXIII antigens was detected using peroxidase-conjugated anti-human immunoglobulins (G+M+A) and a chemiluminescent substrate.

Patient

The initial part of this patient's history was previously reported as acquired FXIII deficiency "in Japanese" by orthopedic surgeons [13]; in brief, our patient with unexplained hemorrhage was admitted to the Department of Orthopedic Surgery of our hospital on Oct 17, 2009 for large intramuscular hematoma in her left thigh and severe anemia. She experienced repeated life-threatening bleeding episodes at multiple sites, including retroperitoneal, intrathoracic, and mediastinal, for approximately 3 weeks. Her FXIII activity was reduced to 10 % of normal despite normal bleeding time, prothrombin time (PT), activated partial thromboplastin time (APTT), and platelet count (Table S1). She received repeated transfusions of red blood cell concentrates (Fig. 1, top), and the arteries flowing the bleeding sites were topically treated using transcatheter arterial embolization on Nov 1 and 7, 2009.

Plasma-derived FXIII-concentrates (1,200 U in 5 vials of Fibrogammin; CSL Behring, Tokyo, Japan) were administered daily starting on Nov 7 (Fig. 1, top) because of her isolated severe FXIII deficiency. On Nov 10, because of her severe hemorrhagic symptoms, she was transferred to the intensive care unit of our hospital, where she suddenly developed shock. She was then intubated and mechanically ventilated. Computed tomography (CT) revealed the presence of cardiac tamponade due to pericardial hemorrhage and an intrathoracic hematoma (Fig. S1 A, B).

To explore the pathogenesis of the patient's isolated acquired FXIII deficiency and determine its ideal treatment modality, the patient was referred to the Japanese Collaborative Research Group (JCRG) for detailed FXIII analyses. After being definitely diagnosed with AH13, she was started on rituximab, an anti-CD 20 monoclonal antibody, 375 mg/m^2/week for 4 weeks from Nov 13 (Fig. 1, top). Hemostatic therapy with FXIII-concentrates was also continued every other day. Despite our extensive search, we could not find any evidence for the underlying diseases of AH13, such as cancer, autoimmune disease, and medication history of isoniazid, antibiotics, and anticonvulsants [8, 9].

Although "clinical remission" was achieved, i.e., the hemorrhage was arrested, the patient's FXIII activity remained low (around 10 % of normal) despite her FXIII antigen level being much higher than normal (>200 %) because of the continued administration of the FXIII-concentrates (Fig. 1, top). These findings suggested the persistence of an FXIII inhibitor. Therefore, she was intravenously given cyclophosphamide 10 mg/kg every other week three times starting in Jan 2010. Since no improvement in FXIII activity was seen, rituximab was added in Feb 2010. However, the combination of these immunosuppressants did not have any immediate effect on FXIII activity (Fig. 1, bottom).

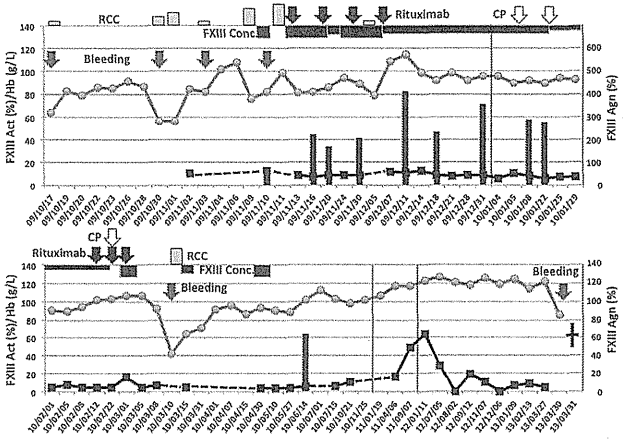

Fig. 1 Clinical course of the present AH13 case. Hemoglobin levels (Hb, *closed circle*) corresponded to bleeding episodes and transfusion of red blood cells (red cell concentrates; RCC) in the opposite directions. Plasma-derived FXIII-concentrates were given at 1,200 U first daily, secondly ever other day, and third every 3 days. The patient underwent immune-suppressive therapies firstly with rituximab and secondly cyclophosphamide (CP) pulse, and thirdly with rituximab. Although FXIII antigen (*filled column*) increased up to 416 % of the normal, FXIII activity (*closed square*) remained low at around 10 % following the replacement therapy with FXIII-concentrates. FXIII activity spontaneously rose and gradually decreased to 5 % 3 days before her hemorrhagic death. *Vertical lines* depict the ends of years

Despite treatment, the patient's FXIII level remained low and her hemoglobin level suddenly dropped to 42 g/L again in Mar 2010. CT imaging revealed an intramuscular hematoma in her left thigh that was successfully controlled by a daily infusion of FXIII-concentrates (Fig. 1, bottom). She underwent no further immunosuppressive treatment, such as steroids, cyclosporine, etc., in order to avoid infection, because of her old age and low capacity for daily living: she was bedridden as well. In addition, there is no gold standard by which AH13 patients are treated. Since her general condition stabilized without further bleeding episodes, she was discharged from the hospital to her home 6 months after admission.

Thereafter, she was regularly followed once a month by her original physician in another hospital. Her FXIII activity level started to spontaneously increase 8 months after the final dose of rituximab, reached 64 % of normal in the following 15 months, and then gradually returned to 10 % of normal after another 10 months (Fig. 1, bottom). Nevertheless, no bleeding episodes were observed for about 3 years.

On the morning of Mar 30, 2013, she was found lying on the floor. She complained of abdominal pain and repeatedly vomited for several hours. When she was transferred to a local hospital by ambulance, there were bruises on her right forehead, left elbow, and left knee. She had tenderness at the left hypochondrium. Laboratory examinations revealed moderate anemia (hemoglobin 85 g/L) and an essentially normal platelet count and PT, and slightly shortened APTT (Table S2). CT scanning revealed a subcortical hemorrhage in the right frontal lobe and a splenic laceration with intraperitoneal hemorrhage (Fig. S1 C, D). She developed shock and died shortly thereafter.

Results and discussion

Laboratory coagulation tests

All coagulation-fibrinolysis factors, except for FXIII were within normal ranges on Nov 2 (Table S1). D-dimer, thrombin–antithrombin complex, and von Willebrand factor antigen levels increased slightly or moderately, according to the patient's severe bleeding condition.

Because the patient's FXIII activity and antigen were 13 and 80 % of the normal values, respectively, on Nov 10, the specific activity was as low as 0.16 (normal value, 1.0). In addition, a 1:1 cross-mixing test between the patient's and a healthy control's plasma clearly demonstrated an "inhibitor" pattern (patient, 19 %; control, 104 %; and mixed, 17 %). All these results suggested the presence of an FXIII inhibitor.

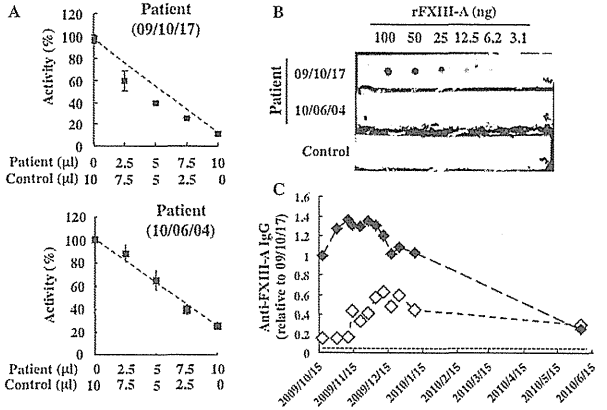

Fig. 2 JCRG analyses of the patient's plasma FXIII and detection of anti-FXIII antibodies. a A 5-step dilution cross-mixing test by an amine incorporation assay was performed using the patient's plasma at the ratios of 0:1, 1:3, 1:1, 3:1, and 1:0 with a normal plasma. The mixed samples showed an "inhibitor" pattern, because there was a downward deviation (Oct. 17, 2009 or hospital day 1). A broken line depicts a theoretical 'deficient' pattern. The patient's plasma of June 4, 2010 demonstrated the deficiency pattern. b A dot blot assay was performed using recombinant FXIII-A (rFXIII-A) at the indicated amounts shown as antigen (ng). The results showed the presence of anti-FXIII-A antibodies (on Oct. 17, 2009). The negative control stands for healthy individual's plasma. The patient's plasma of June 4, 2010 showed a negative result. c After the immunosuppressive therapy, her anti-FXIII-A immunoglobulin G (IgG) had significantly decreased, judging from our ELISA results. Although the dot blot assay did not detect anti-FXIII-A autoantibodies in her sample of June 4, 2010 (b), a small amount of anti-FXIII-A IgG was still clearly detected by the ELISA (*filled diamonds*). A similar amount of anti-FXIII-A IgG bound to own FXIII-A was also detected by the ELISA without adding rFXIII-A (*open diamonds*). A dashed line represents an average level of normal controls

Experimental FXIII tests

The patient's plasma on Oct 17 (hospital day 1) was analyzed by the JCRG in detail; the FXIII-A antigen was 23 % of normal and the FXIII amine incorporation activity was 12 %, thus its specific activity was 0.47 (Table S3). Her FXIII-B antigen was 85 % of normal. Although her FXIII activity was extremely low during this acute stage, both FXIII-A and FXIII-B proteins retained their normal molecular weights as shown by western blotting (Fig. S2 A).

Our 5-step dilution cross-mixing test of the patient's plasma demonstrated a concave "inhibitor" pattern (Fig. 2a, top). An in-house dot blot test using rFXIII-A, rFXIII-B, and rA_2B_2 tetramer detected anti-FXIII-A autoantibodies (Fig. 2b, top; Fig. S2 C). In addition, our ELISA method clearly detected anti-FXIII-A antibodies (Fig. 2c). Accordingly, she was definitely diagnosed with AH13. A fibrin cross-linking test also visualized a drastic retardation in γ-chain dimerization and the absence of α-chain polymerization (Fig. S2 B).

Approximately 3 months after the final administration of rituximab, the patient's plasma showed a straight "deficient" pattern in the 5-step dilution cross-mixing test (Fig. 2a), suggesting that her FXIII inhibitor had virtually disappeared. This finding agreed with the fact that the dot blot analysis did not detect anti-FXIII-A antibodies. This partial recovery may be associated with the slower effect of rituximab [14] or spontaneous antibody regression [15].

Our ELISA, however, detected small amounts of total and FXIII-A-bound anti-FXIII-A antibodies (Fig. 2c). These findings are quite in agreement with the fact that the patient had 64 and 6 % of FXIII antigen and activity, respectively (Fig. 1, bottom), indicating the presence of the FXIII/13 antigen–antibody complex in the patient's sample.

Extended coagulation screening tests (Table S4) were also conducted by the JCRG to take an overall look at the current status of coagulation–fibrinolysis system in this AH13 case. All test results were consistent with those discussed above.

It was interesting to note that the patient's FXIII activity transiently increased in late 2011 and gradually decreased in early 2012: It may be attributable to the rather late effect of rituximab because it may require more time than other regimens [14]. Alternatively, the amount of her