6 第XIII因子製剤

a 製剤の種類

わが国ではフィブロガミン®Pが販売されている．本剤は米国，ドイツ，オーストリアで採血された健常者血液から精製された製品でウイルス不活化のための加熱処理が施されている．

b 適応

第XIII因子はトロンビンによって活性化第XIII因子となり，フィブリン分子間に架橋を形成して安定化フィブリンとし，さらにα_2-plasmin inhibitorをフィブリンに結合させて線溶に対する抵抗性を付与する．また細胞外マトリックスの構成成分であるコラーゲンやフィブロネクチンを重合させる．これらの作用は止血や創傷治癒に深く関与している．第XIII因子の半減期は約10日ときわめて長い．

フィブロガミン®Pは先天性第XIII因子欠乏症，術後縫合不全・瘻孔，アレルギー性紫斑病（Schönlein-Henoch紫斑病，IgA血管炎）に保険適用がある．先天性第XIII因子欠乏症の多くは第XIII因子活性が1%未満であり，出産時の臍帯出血が特徴的で新生児期に頭蓋内出血をきたしやすい．第XIII因子が10～20%以上あれば十分な止血効果がある．第XIII因子は手術や肝疾患などでしばしば低下するが1%未満になることはなく，出血症状を呈することはほとんどない．しかし，第XIII因子に対する自己抗体が産生されて第XIII因子活性が著減し，後天性第XIII因子欠乏症と称される出血性疾患が報告されている．本症では第XIII因子活性以外の血液凝固検査で異常がみられないことから見逃されやすい．

縫合不全での適応は低アルブミン血症がなく第XIII因子が70%以下に低下している場合である．アレルギー性紫斑病での適応は腹部症状または関節症状があり，第XIII因子が90%以下に低下している場合である．いずれの場合でも本剤はヒト血液を原材料としている製剤であり，他の治療手段も十分考慮したうえで慎重に使用する．

文　献

1) Manco-Johnson MJ, et al：Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. N Engl J Med 357：535-544, 2007
2) Yasaka M, et al：Correction of INR by prothrombin complex concentrate and vitamin K in patients with warfarin related hemorrhagic complication. Thromb Res 108：25-30, 2002
3) Yasaka M, et al：Optimal dose of prothrombin complex concentrate for acute reversal of oral anticoagulation. Thromb Res 115：455-459, 2005
4) Eerenberg ES, et al：Reversal of rivaroxaban and dabigatran by prothrombin complex concentrate. A randomized, placebo-controlled, crossover study in healthy subjects. Circulation 124：1573-1579, 2011
5) Leissinger C, et al：Anti-inhibitor coagulant complex prophylaxis in hemophilia with inhibitors. N Engl J Med 365：1684-1692, 2011

Development of exogenous FVIII-specific inhibitor in a mild haemophilia patient with Glu272Lys mutation

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Mild haemophilia A is commonly caused by a missense mutation in the FVIII gene responsible for a decrease in FVIII:C to between 5 and 40 (IU dL^{-1}) [1]. Although such a significant residual amount of FVIII antigen is likely to prevent the immune response to exogenously administered FVIII, 3–13% of patients with mild haemophilia can develop an inhibitor antibody, which usually neutralizes not only exogenous but also endogenous FVIII [2,3]. The risk of inhibitor development in mild haemophilia increases with age, at a median of 66 years, which is a distinct feature from the case of severe haemophilia [3].

Genetic risk factors for the development of inhibitor in mild haemophilia have been explored, and the Arg593Cys and Trp2229Cys mutations of FVIII were identified as a risk factor of inhibitor development [4,5]. Several other mutations have been also reported in mild haemophiliacs with inhibitors [2,5,6]. How-

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ever, there is no report on the association of genotypes with immunological characteristics of inhibitors. We describe here how a mild haemophilia patient carrying the Glu272Lys mutation developed an inhibitor, which neutralized exogenous wild-type FVIII, but not autologous mutant FVIII.

A 73-year-old man was diagnosed with mild haemophilia A in his teens after he had a difficulty in haemostasis after traumatic injury, which resolved without any treatment. His cousin also suffered from mild haemophilia A. The FVIII:Ag and FVIII:C were 38% and 13% respectively. He had no bleeding episode requiring FVIII replacement therapy until the age of 70. At the age of 71, he was given recombinant FVIII (rFVIII) products for the first time because of massive bleeding following transurethral resection of the prostate. At the age of 72, he underwent appendectomy and received rFVIII for the second time for 7 days. The FVIII:C was increased following rFVIII infusion as expected, and an inhibitor detection test (Bethesda assay) was negative.

At the age of 73, he had a left shoulder joint pain and swelling. Arthrocentesis showed yellow purulent fluid with *Staphylococcus aureus*. He was given antibiotics and he underwent arthroscopic excision of a synovial membrane and joint washing to treat the joint abscess under the third replacement therapy for 4 days. No bleeding complications were noted during surgery. However, 12 days after surgery, he had pain and swelling in his left shoulder. The relapse of the joint abscess was initially suspected and arthrocentesis was performed, which showed fresh bleeding in the joint without bacterial infection. We immediately measured FVIII:C after administration of rFVIII, and found it to be 13.8%, indicating no increase in FVIII:C above his basal level of FVIII:C. The Bethesda assay showed 4.2 BU mL^{-1} of FVIII inhibitor. He was administered a recombinant FVIIa product (NovoSeven®; Novo Nordisk, Tokyo, Japan), which stopped the haemorrhage. Then he had no bleeding episode requiring FVIII replacement therapy. The titre of inhibitor was spontaneously decreased and disappeared 6 months later.

The patient had a significant residual FVIII:C in the presence of a relatively high titre of inhibitor, suggesting a type II inhibitor that was often seen in acquired haemophilia [7]. However, his inhibitor exhibited a linear dose–response relationship characteristic for type I inhibition kinetics when his plasma was mixed with normal pooled plasma (Fig. 1). To explain the discrepancy between the significant residual FVIII:C and the presence of type I inhibitor in this patient, we hypothesized that the patient's inhibitor did not neutralize autologous mutant FVIII but wild-type FVIII. To test this, we examined whether the patient's inhibitor could neutralize the endogenous mutant FVIII or not. The patient's plasma containing FVIII inhibitor

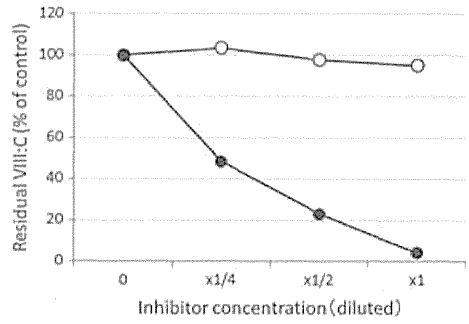

Fig. 1. Differential effect of patient's FVIII inhibitor on exogenous wild-type FVIII and autologous mutant FVIII. The patient's plasma containing an inhibitor was treated at 56°C for 30 min to eliminate residual VIII:C. The treated plasma (inhibitor concentration: x1) was diluted with a HEPES buffer by two and four times, and incubated with normal pooled plasma (closed circle), patient's plasma stored before inhibitor development (open circle), or buffer (inhibitor concentration: 0) at 37°C for 2 h. The residual VIII:C was measured by one-stage clotting assay and expressed as percentages of the value for each plasma incubated with buffer.

was treated at 56°C for 30 min to eliminate the residual endogenous FVIII:C. The mixture of the treated plasma and the patient's plasma stored at −80°C before the appearance of the inhibitor were subjected to the Bethesda assay. The patient's inhibitor in the treated plasma did not neutralize mutant FVIII:C in the stored plasma at all while it neutralized wild-type FVIII:C in normal pooled plasma completely (Fig. 1). This differential effect of the patient's inhibitor was specific because another inhibitor from a severe haemophiliac neutralized both the wild-type and mutant FVIII:C completely. These results indicate the patient's inhibitor is specifically directed against exogenous wild-type FVIII. We further examined the epitope of the inhibitor by means of immunoblotting. The inhibitor recognized both A2 and A3-C1-C2 (light chain) domains of wild-type FVIII (Fig. 2).

We attempted to identify a mutation of FVIII gene responsible for mild haemophilia in this patient. The FVIII gene entire coding regions and exon/intron boundaries were amplified by PCR with appropriate primers. The PCR products were purified and directly sequenced using a 3500 Genetic Analyzer (Applied Biosystems, Tokyo, Japan). The genetic study was approved by the Ethics Committee of Ehime University. Sequence analysis of FVIII in this patient showed an 871G > A alteration (Glu272Lys) in exon 7 of the FVIII gene. The patient's cousin with mild haemophilia had the same mutation.

This study shows that a patient with mild haemophilia, who carried the Glu272Lys mutation of FVIII, developed an inhibitor. According to the HAMSTeRS worldwide database for mutations in haemophilia A (http://hadb.org.uk), this is the first report that the Glu272Lys mutation is associated with inhibitor development although this mutation has been found to be causative for mild haemophilia. In addition, this

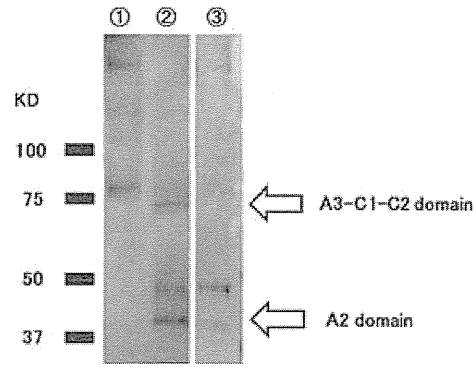

Fig. 2. Inhibitor epitope analysis. The wild-type FVIII derived from a recombinant FVIII product was cleaved with (lanes 2, 3) or without (lane 1) thrombin and then subjected to Western blotting with patient's plasma containing an inhibitor (lanes 1, 2) or normal plasma (lane 3). The immunoreactive bands were detected by Amersham ECL Chemilunescence (GE Healthcare). The two strong bands showing the A2 domain and light chain (A3-C1-C2 domain) of FVIII were consistently obtained while the 50 KD band corresponding to the A1 domain was also seen in the negative control blot with normal plasma. A representative blot of the three experiments is shown.

study shows that the inhibitor recognized exogenous wild-type FVIII but not autologous mutant FVIII. These findings suggest a possible association of the Glu272Lys mutation with the exogenous FVIII-specific inhibitor.

Previous studies have shown that certain mutations of the FVIII gene in mild haemophilia have a greater risk for inhibitor development. Missense mutations introducing a cysteine residue may alter disulphide bridge formation, leading to a gross aberrant conformation. The mutations including Arg531Cys [6], Arg593Cys [4,5], Tyr2105Cys [4] and Trp2229Cys [4] have been reported to be associated with inhibitor development. Among them, Arg593Cys was identified as a 10-fold increased risk of developing inhibitors [5]. However, inhibitor development is not limited to patients with Cys substitution mutations. The Asn618-Ser, Pro1761Gln, Arg2150His and Glu2228Asp mutations have been found in mild haemophiliacs who developed an inhibitor [8]. In this study, we extended the list of genetic mutations associated with FVIII inhibitor to Glu272Lys.

Besides the genetic risk factor, some environmental factors including intensive FVIII treatment, age at the first exposure and the number of peak treatment are associated with inhibitor development in mild haemophilia [3]. Our patient had three intensive FVIII treatments since the age of 71. The inhibitor developed after the third intensive therapy for surgery during bacterial infection. The risk of inhibitor development after surgery has been demonstrated in both severe and mild haemophilia [5,8]. The presence of infection during the FVIII treatment may enhance the immune response to exogenous FVIII through the infection-triggered inflammatory responses, leading to development of inhibitor antibodies. A patient with mild haemophilia who was excised of an infected femur head was reported to develop an inhibitor after intensive FVIII treatment [8]. Further research is needed to determine whether infection or surgery for infected tissues during intensive FVIII treatment is a risk for inhibitor development.

The FVIII infused into patients with haemophilia is recognized as being different from autologous one, generating an alloantibody. We, therefore, anticipated that the inhibitor developed in our patient was directed against the A1 domain in which the mutated residue, Glu272, was located. Unexpectedly, the inhibitor did not recognize the A1 domain but the A2 and A3-C1-C2 domains. Previous studies have demonstrated that the epitopes of almost all FVIII inhibitors were located in the A2 and/or A3-C1-C2 domains, suggesting a high immunogenicity of these domains [7]. There have been few reports demonstrating alloantibodies to the A1 domain [9]. In the tertiary structure of the FVIII protein, the Glu272 is in close proximity to the residues 482–501 in the A2 domain which constitutes a hot spot for the epitopes of inhibitors [10]. The Glu272Lys mutation may induce conformational changes in the A2 domain of the endogenous mutant FVIII that allow to develop an inhibitor against the A2 domain of exogenous wild-type FVIII.

The majority of inhibitors in patients with mild haemophilia have been reported to cross-react with autologous FVIII, resulting in little or no residual FVIII:C [4,5]. In this respect, our case represents a minor phenotype. Similar cases have been reported in mild haemophilia patients carrying Glu2228Asp and Try2229Cys mutations [4,5]. Although these cases showed that the FVIII:C did not change after the appearance of inhibitors, characterization of inhibitors has not been reported. Our present report shows only a single case. It remains to be determined whether a specific mutation is associated with development of inhibitor that does not cross-react with autologous mutant FVIII. In patients with a non-cross-reactive inhibitor, DDAVP may be an effective therapeutic option although we failed to try DDAVP in our patient.

In conclusion, this study shows that a mild haemophilia patient with the Glu272Lys mutation of FVIII developed an exogenous FVIII-specific inhibitor. Data regarding the link between genotypes and characteristics of inhibitors should be accumulated in mild haemophilia. Such information would contribute to more appropriate patient management and help better define the molecular basis of inhibitor development in mild haemophilia.

Author contributions

J. Yamanouchi treated the patient, performed experiments and wrote the manuscript. T. Hato designed the study and wrote the

manuscript. T. Niiya performed the Bethesda assay. Y. Sato performed the sequence analysis. S. Onishi performed the Western blotting assay. M. Yasukawa supervised the research and reviewed the manuscript. All authors have approved the final submitted version.

Disclosures

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

References

1 Franchini M, Favaloro EJ, Lippi G. Mild hemophilia A. *J Thromb Haemost* 2010; 8: 421–32.

2 Hay CR. Factor VIII inhibitors in mild and moderate-severity haemophilia A. *Haemophilia* 1998; 4: 558–63.

3 Mauser-Bunschoten EP, Den Uijl IE, Schutgens RE, Roosendaal G, Fischer K. Risk of inhibitor development in mild haemophilia A increases with age. *Haemophilia* 2012; 18: 263–7.

4 Hay CR, Ludlam CA, Colvin BT *et al.* Factor VIII inhibitors in mild and moderate-severity haemophilia A. *Thromb Haemost* 1998; 79: 762–6.

5 Eckhardt CL, Menke LA, Van Ommen CH *et al.* Intensive peri-operative use of factor VIII and the Arg593 > Cys mutation are risk factors for inhibitor development in mild/moderate hemophilia A. *J Thromb Haemost* 2009; 7: 930–7.

6 Cid AR, Casana P, Cabrera N, Haya S, Cortina V, Azmar JA. Inhibitor development in one patient and laboratory discrepancies in several families with both mild haemophilia and Arg531Cys mutation. *Haemophilia* 2007; 13: 206–8.

7 Shima M. Characterization of factor VIII inhibitors. *Int J Hematol* 2006; 83: 109–18.

8 Eckhardt CL, Mauser-Bunschoten EP, Peters M, Leebeek FW, van der Meer FJ, Fijnvandraat K. Inhibitor incidence after intensive FVIII replacement for surgery in mild and moderate haemophilia A: a prospective national study in the Netherlands. *Br J Haematol* 2012; 157: 747–52.

9 Shibata M, Shima M, Morichika S *et al.* An alloantibody recognizing the FVIII A1 domain in a patient with CRM reduced haemophilia A due to deletion of a large portion of the A1 domain DNA sequence. *Thromb Haemost* 2000; 84: 442–8.

10 d'Oiron R, Pipe SW, Jacquemin M. Mild/moderate haemophilia A: new insights into molecular mechanisms and inhibitor development. *Haemophilia* 2008; 14: 138–46.

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LETTER TO THE EDITOR

Severe immune thrombocytopenia secondary to Waldenström's macroglobulinemia with anti-GPIb/IX monoclonal IgM antibody

Jun Yamanouchi · Takaaki Hato · Toshiyuki Niiya · Taichi Azuma · Masaki Yasukawa

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Dear Editor,

Waldenström's macroglobulinemia (WM) is a lymphoproliferative disorder characterized by infiltration of small lymphocytes and plasmacytoid cells into bone marrow and by serum IgM monoclonal gammopathy [1, 2]. The paraprotein may include an autoantibody resulting in autoimmune complications in 5–16 % of patients with WM [3, 4]. A few reports have referred to the mechanism of immune thrombocytopenia (ITP) associated with WM, and platelet-associated IgM (PA-IgM) and IgG (PA-IgG) have been shown to be a possible cause [4, 5]. However, whether monoclonal IgM in WM induces ITP is poorly understood. We report here a patient with WM and thrombocytopenia whose IgM recognized GPIb/IX on the surface membrane of platelets and inhibited GPIb-mediated platelet aggregation.

A 68-year-old male was referred to our department because of extensive purpura and macrohematuria. His blood counts revealed 5.3 g/dL hemoglobin, 1.4×10^9/L white blood cells, and 6.0×10^9/L platelets. Platelet size was normal. Serum levels of IgM, IgG, and IgA were 1,980, 1,470, and 55 mg/dL, respectively. Immunoelectrophoresis revealed that almost all IgM was monoclonal. Bone marrow-nucleated cells comprised 44.2 % lymphoid cells and 0.6 % plasmacytoid cells, which were positive for CD10, CD19, CD20, CD38, and SmIg-κ, but negative for CD5 and CD23. He was diagnosed as having WM and treated with cyclophosphamide. He was refractory to platelet transfusion and died of brain hemorrhage 1 week later.

Because WM-induced ITP was suspected, we examined the effect of his IgM on platelets. Flow cytometry assay showed that his IgM bound to normal platelets whereas his IgG did not. To identify the platelet antigen recognized by IgM, we employed a PakAuto assay. This assay enables us to characterize the antigen of platelet-bound Ig. However, we failed to obtain Ig eluted from patient's platelets because of very low platelet count. Then the patient's serum was incubated with normal platelets and the platelet-bound Ig was eluted in order to concentrate platelet-specific Ig from the patient's whole serum. Furthermore, we employed alkaline phosphatase-conjugated goat anti-human IgG and IgM (BioFX, Owings Mills, MD, USA) as secondary antibody to detect IgG and IgM separately. We confirmed that these antibodies produced efficient and specific signals when the positive and negative controls in the PakAuto assay kit were employed. Using this modified assay, we found that the eluted IgM bound to the immobilized platelet membrane GPIb/IX, but not to GPIIb/IIIa or GPIa/IIa (Fig. 1). The eluted IgG did not bind to any type of GP.

Then we examined the effect of patient's IgM on GPIb-mediated platelet aggregation. When the patient's plasma was incubated with normal washed platelets, 1.2 mg/mL ristocetin-induced platelet aggregation was inhibited significantly although the expression of GPIb on the platelets was normal.

These results suggest that the patient's monoclonal IgM derived from WM has antiplatelet activity against GPIb/IX. This activity leads to ITP and platelet dysfunctions, both of

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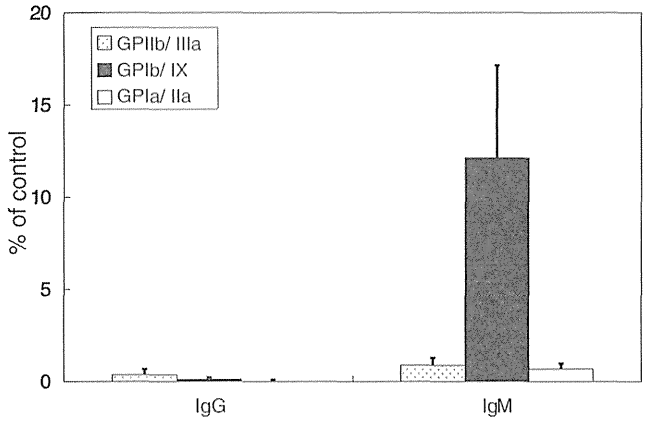

Fig. 1 Determination of platelet membrane glycoprotein recognized by patient's serum immunoglobulin. Normal washed platelets were incubated with the patient's serum. Acid eluates from the platelets were added to microwell strips coated with murine monoclonal antibody-immobilized platelet GPs (*IIb/IIIa*, *Ib/IX*, and *Ia/IIa*), and alkaline phosphatase-labeled anti-human IgG or IgM were added to the wells followed by enzyme substrate PNPP. The optical density of wells was read at 405 nm and the binding reactivity was expressed as percentages of the value for the positive control serum included in the PakAuto assay kit

which may give rise to severe hemorrhagic symptoms in this patient. The patient's IgG may be also involved in the pathogenesis of ITP because it remains to be determined whether patient's IgG binds on the surface of autologous platelets.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Gertz MA (2012) Waldenström macroglobulinemia: 2012 update on diagnosis, risk stratification, and management. Am J Hematol 87:503–510
2. Dimopoulos MA, Gertz MA, Kastritis E, Garcia-Sanz R, Kimby EK, Leblond V, Fermand JP, Merlini G, Morel P, Morra E, Ocio EM, Owen R, Ghobrial IM, Seymour J, Kyle RA, Treon SP (2009) Update on treatment recommendations from the Fourth International Workshop on Waldenström's Macroglobulinemia. J Clin Oncol 27:120–126
3. Kyrtsonis MC, Vassilakopoulos TP, Angelopoulou MK, Siakantaris P, Kontopidou FN, Dimopoulou MN, Boussiotis V, Gribabis A, Konstantopoulos K, Vaiopoulos GA, Fessas P, Kittas C, Pangalis GA (2001) Waldenström's macroglobulinemia: clinical course and prognostic factors in 60 patients. Experience from a single hematology unit. Ann Hematol 80:722–727
4. Owen RG, Lubenko A, Savage J, Parapia LA, Jack AS, Morgan GJ (2001) Autoimmune thrombocytopenia in Waldenström's macroglobulinemia. Am J Hematol 66:116–119
5. Yamamoto K, Nagata K, Hamaguchi H (2002) Elevated platelet-associated IgG in immune thrombocytopenic purpura associated with Waldenström's macroglobulinemia. Am J Hematol 69:85–86

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LETTER TO THE EDITOR

Dramatic and prompt efficacy of *Helicobacter pylori* eradication in the treatment of severe refractory iron deficiency anemia in adults

Jun Yamanouchi · Taichi Azuma · Yoshihiro Yakushijin · Takaaki Hato · Masaki Yasukawa

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Dear Editor,

Iron deficiency anemia (IDA) is one of the most common nutritional diseases worldwide. The basic treatment for IDA is oral iron medication; however, some patients with IDA are occasionally refractory to this type of therapy [1]. Recently, the role of *Helicobacter pylori* in the etiology of IDA has become a focus of considerable interest [2, 3]. Although the association of *H. pylori* with IDA has been examined extensively, most studies have focused on mild IDA in school-aged children and young people [4–6]. Here, we report two adult cases of severe IDA refractory to oral iron therapy in which the anemia was dramatically and promptly improved by *H. pylori* eradication (Fig. 1).

Case 1 A 73-year-old male was referred to our hospital because of severe anemia. He had been receiving oral iron (100 mg/day) therapy for more than 6 months, but his anemia had not improved significantly. Laboratory examinations revealed severe IDA (red blood cell count 269×10^{10}/l, hemoglobin 4.7 g/dl, hematocrit 17.5 %, mean corpuscular volume 65.1 fl, mean corpuscular hemoglobin concentration 17.5 pg, serum iron 8 μg/dl, serum ferritin 4.7 ng/ml, unsaturated iron binding capacity 487 μg/dl). Endoscopic examination of the upper and lower gastrointestinal (GI) tract revealed mild atrophic gastritis with *H. pylori* infection, but no bleeding was detected in any part of the GI tract. A stool occult blood test gave a negative result. CT examination also revealed no abnormalities. Because oral iron therapy had failed in this patient, iron was administered intravenously, and this resulted in a gradual increase in the hemoglobin level. *H. pylori* eradication was performed using a standard regimen of amoxicillin, clarithromycin, and lansoprazole. Eradication was successful, and iron was then administered orally (100 mg/day). In contrast to the lack of response to oral iron therapy before *H. pylori* eradication, the patient's hemoglobin level promptly increased to normal. Since discontinuation of iron therapy, IDA has not recurred for more than 3 years.

Case 2 A 36-year-old male was referred to our hospital because of severe IDA that was refractory to oral iron therapy. Laboratory examinations revealed the following values: red blood cell count 235×10^{10}/l, hemoglobin 4.6 g/dl, hematocrit 17.0 %, mean corpuscular volume 72.3 fl, mean corpuscular hemoglobin concentration 19.6 pg, serum iron 10 μg/dl, serum ferritin 5.3 ng/ml, and unsaturated iron binding capacity 453 μg/dl. Endoscopic examination of the whole GI tract and CT scan revealed no abnormalities. Since *H. pylori* infection was detected, *H. pylori* eradication was performed. Similarly to the clinical course in case 1, IDA was promptly improved by oral iron administration. This patient also has shown no recurrence of IDA for more than 2 years after withdrawal of iron therapy.

Although an association between *H. pylori* infection and IDA has been established, it is still unknown how *H. pylori* mediates the pathogenesis of IDA. However, a number of possible mechanisms have been proposed, including impairment of gastric acidity by *H. pylori* infection [7]. Recently, it has been reported that *H. pylori* directly perturbs iron trafficking in epithelial cells [8, 9]. That is, CagA of *H. pylori* is able to alter the internalization, intracellular transport, and polarity of the transferrin/transferrin receptor iron uptake system [9]. The prompt improvement of iron adsorption observed after *H. pylori* eradication in the present two cases strongly

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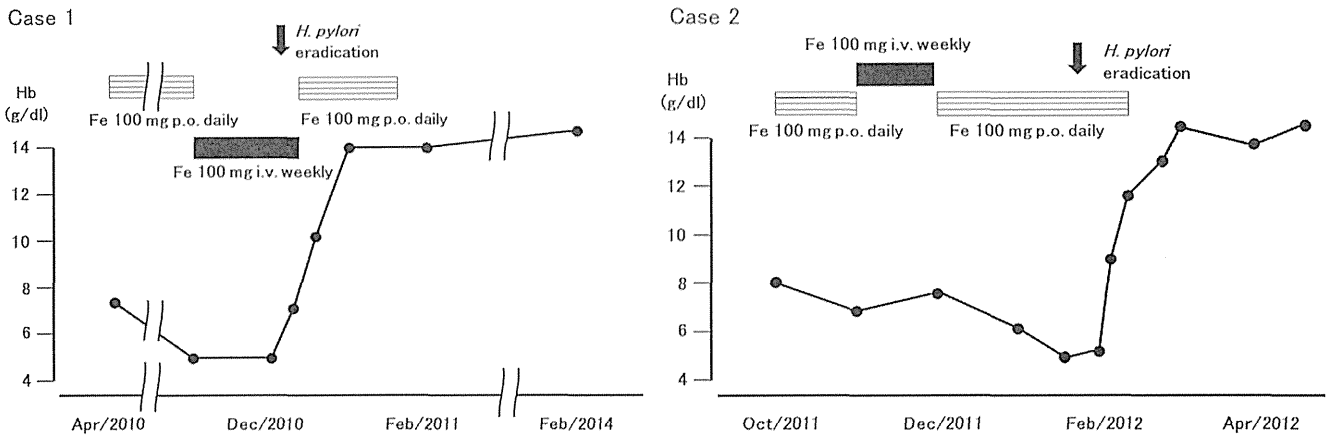

Fig. 1 Clinical courses of cases 1 and 2. Refractory IDA was promptly improved by oral iron administration after *H. pylori* eradication and did not recur after withdrawal of iron therapy in both cases

suggests a direct interaction between *H. pylori* and iron transport. As it has also been reported that IDA accelerates *H. pylori*-induced carcinogenesis [10], *H. pylori* eradication is highly recommended in patients with IDA.

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Hershko C, Camaschella C (2014) How I treat unexplained refractory iron deficiency anemia. Blood 123:326–333
2. Muhsen K, Cohen D (2008) *Helicobacter pylori* infection and iron stores: a systematic review and meta-analysis. Helicobacter 13: 323–340
3. Cardenas VM, Mulla ZD, Ortiz M, Graham DY (2006) Iron deficiency and *Helicobacter pylori* infection in the United States. Am J Epidemiol 163:127–134
4. Baggett HC, Parkinson AJ, Muth PT, Gold BD, Gessner BD (2006) Endemic iron deficiency associated with *Helicobacter pylori* infection among school-aged children in Alaska. Pediatrics 117:e396–404
5. Muhsen K, Barak M, Henig C, Alpert G, Ornoy A, Cohen D (2010) Is the association between *Helicobacter pylori* infection and anemia age dependent? Helicobacter 15:467–472
6. Azab SF, Esh AM (2013) Serum hepcidin levels in *Helicobacter pylori*-infected children with iron-deficiency anemia: a case–control study. Ann Hematol 92:1477–1483
7. Annibale B, Capurso G, Lahner E, Passi S, Ricci R, Maggio F, Delle Fave G (2003) Concomitant alterations in intragastric pH and ascorbic acid concentration in patients with *Helicobacter pylori* gastritis and associated iron deficiency anaemia. Gut 52:496–501
8. Lee JH, Choe YH, Choi YO (2009) The expression of iron-repressible outer membrane proteins in *Helicobacter pylori* and its association with iron deficiency anemia. Helicobacter 14:36–39
9. Tan S, Noto JM, Romero-Gallo J, Peek RM Jr, Amieva MR (2011) *Helicobacter pylori* perturbs iron trafficking in the epithelium to grow on the cell surface. PLoS Pathog 7:e1002050
10. Noto JM, Gaddy JA, Lee JY et al (2013) Iron deficiency accelerates *Helicobacter pylori*-induced carcinogenesis in rodents and humans. J Clin Invest 123:479–492

実地医家が身につけるべき止血異常の基本的治療法とその活用

止血異常に対する輸血療法の基本

—適切な血小板製剤，新鮮凍結血漿の入手と適切な使いかた—

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はじめに

血液検査で血小板もしくは凝固系に重大な異常がみられると補充療法としての血小板輸血もしくは新鮮凍結血漿の輸注がしばしば考慮される．これら輸血用血液製剤は止血血栓の形成に必要な原材料を含んでいることから適切な患者に必要な量が投与されれば迅速な止血効果を期待することができる．その反面，不適切な投与がなされるとかえって病態を悪化させてしまう場合もある．本稿では止血検査値からみた血小板製剤および新鮮凍結血漿の投与基準と病態ごとの注意点について述べる．

血小板輸血

1. 適切な血小板輸血トリガー値

予防的血小板輸血の開始基準となる血小板数値(輸血トリガー値)に関する無作為対照試験が急性白血病・造血幹細胞移植患者を対象として複数行われ，いずれの試験においても2万/μlから1万/μlにトリガー値を下げても出血予防効果は変わらないことが証明されてきた[1]．ただし注意が必要な点は，発熱，軽微でも新たな出血症状の出現，または侵襲的処置前のいずれかに該当する患者は1万群でもそれ以上の値で輸血が行われたということである．このようなケースは日常臨床ではかなりの割合を占めるが，これら臨床試験でも28～49%に及んでいた．すなわち，輸血トリガー値1万/μlの適応となるのは発熱や新たな紫斑のない安定した患者であり，日本を含め世界各国の輸血ガイドラインにこの付帯条件が記されていて，すべての患者でトリガー値1万/μlを遵守せよとの内容ではない．またわが国では原則として血小板製剤を血液センターに予約しておく体制であるために，血小板がトリガー値に到達しても当日中に血小板製剤を入手できるとは限らない．このため，わが国では2万/μl程度を目標に輸血することはやむを得ない．なお再生不良性貧血や骨髄異形成症候群のような慢性的に血小板が減少している患者では血小板数を5,000/μl以上に保てば出血症状をコントロールできることが観察研究で示されている．

2. 観血的処置前の予防的血小板輸血

手術や観血的処置の前に行われる予防的輸血では，血小板数5万/μlを目標に輸血が行われている．肝生検では3万/μl，中心静脈カテーテル挿入では2万/μl，腰椎穿刺では1万/μlの基準で問題ないとの報告があるが，無作為対照試験はまだ実施されていない．実際のところ，観血的処置局所からの出血は，血小板数よりも処置手技の拙劣によってより大きく左右されるので，血小板輸血よりも術者にこだわった方がよい．

3. 治療的血小板輸血

治療的輸血は消化管，肺，脳からの出血のような重篤な出血に対して行うものを指す．高度の血小板減少で重篤な臓器出血をきたすことはあるが，多くは炎症や抗癌剤による局所組織障害が出血の要因になっており，血小板輸血だけでなく，これら要因に対する処置が重要となる．治療的輸血では血小板数5万/μlを目標と

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- 予防的血小板輸血のトリガー値は臨床症状に応じて1～2万/μlの幅がある.
- 手術や観血的処置前には血小板数5万/μlを目標に血小板輸血を行う.
- 予防的血小板輸血を避けなければならない血小板減少症が存在する.
- 血小板輸血不応患者でHLA抗体が陽性ならHLA適合血小板を輸血する.

した輸血が経験的に行われている.

4. トリガー輸血は本当に有用か

血小板数が出血リスクのマーカーではあるものの，出血症状が血小板数単独によって規定されているわけでないことは日常臨床からも明らかであり，血小板数のみを指標とした予防的輸血が適切な手段であるかどうかはわからない．最近，造血器腫瘍患者をトリガー値1万/μlでの予防的輸血群と血小板数にこだわらず出血症状を呈したときのみ輸血する治療的輸血群に無作為に振り分けて輸血量と出血症状を評価する二つの臨床試験が報告された[2, 3]．両試験とも治療的輸血群の出血エピソードは予防的輸血群よりも多く，造血器腫瘍患者への予防的輸血の有用性が確認されたといえる(図1)．

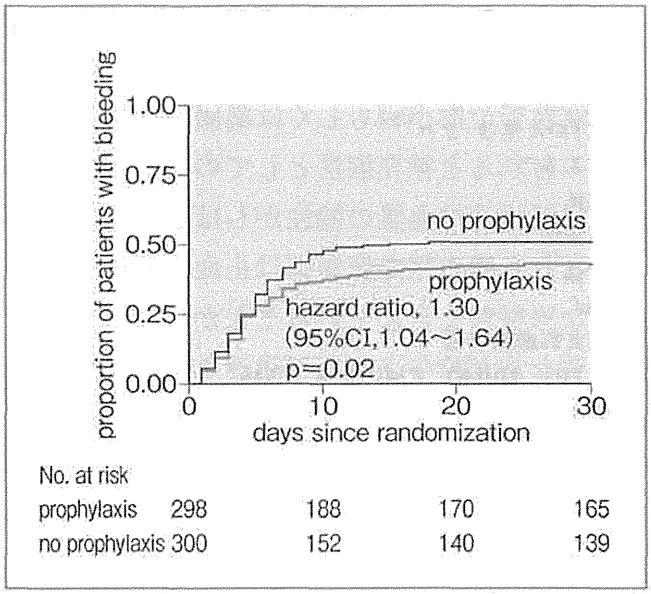

図1 造血器腫瘍患者における予防的血小板輸血の有無における出血頻度

(文献3)より引用)

5. 血小板輸血禁忌の病態

血栓性血小板減少性紫斑病thrombotic thrombocytopenic purpura(TTP)，溶血性尿毒症症候群hemolytic uremic syndrome(HUS)およびヘパリン起因性血小板減少症heparin-induced thrombocytopenia(HIT)では，血小板減少の原因が血小板血栓の多発による消費にあるので，血小板輸血は病態を悪化させるおそれがある．実際，血小板輸血後にTTP，HUSもしくはHIT症状が悪化した症例が報告されている．しかし，手術や重篤な出血のために血小板輸血をやむを得ず行った患者で意外と病態は悪化せず，止血効果が得られてむしろ有用であったとの報告もあり，今後の検証が必要である．ただし，少なくともこれらの患者への予防的輸血は有益性がなく避けるべきである．

6. 血小板輸血不応状態

血小板輸血を行っても予期したほどの血小板数増加が得られない場合を血小板輸血不応状態と呼んでいる．不応状態に陥る原因には，免疫性と非免疫性の要因があり，免疫性要因のほとんどはHLA抗体である(表1)．HLA抗体が検出された場合はHLA適合血小板を輸血する．HLA適合血小板の輸血によって60～70%の患者で血小板数の上昇が期待できる．しかし，限られた登録ドナーから化学療法のサイクルごとにタイミングよくHLA適合血小板を供給し続けることは困難を極め，血小板減少時期を予測してオーダーせざるを得ない．

HLA適合血小板を投与しても血小板数の上昇が得られない場合は非免疫性要因の影響を考

■ FFP の投与前には凝固検査(PT, APTT)を行って明らかな凝固異常のあることを確認する.
■ 凝固検査異常があっても出血症状がなければ FFP の予防的投与はしない.
■ FFP の投与量は凝固因子の上昇と輸注量のバランスに注意して決めるが, 通常 10 ml/kg が適切である.

表 1　血小板輸血不応症の原因

- 免疫性
  - 抗 HLA 抗体(多い)
  - 抗 HPA 抗体(まれ)
- 非免疫性
  - 発熱
  - 感染症
  - 脾腫
  - DIC
  - 造血幹細胞移植
    - TBI, GVHD, CMV 感染, TMA
  - 薬剤性
    - アムホテリシン B, バンコマイシンなど

表 2　FFP 投与開始基準

A. 出血症状
1. 活動性の出血症状(自然出血, 外傷出血)
2. 観血的処置に伴う局所出血の危険

B. 凝固検査異常
1. プロトロンビン時間(PT)　30% 以下(INR 2.0 以上)
2. 活性化部分トロンボプラスチン時間(APTT)　各医療機関における基準値上限の 2 倍以上
3. フィブリノゲン値　100 mg/dl 未満

上記 A の 1 項目かつ B の 1 項目を満たす場合に FFP の投与を開始する.
(厚生労働省薬事・食品衛生審議会血液事業部会適正使用調査会：血液製剤の使用指針(改定版), 2009 第 4 版より改変引用)

える(表 1). 血小板数の増加が得られないにもかかわらず通常の血小板製剤を輸血し続けることが止血に有効であるのか否かは明らかでなく, 欧米のガイドラインでは行うべきでないとしている.

新鮮凍結血漿 fresh frozen plasma (FFP)輸注

1. FFP の投与基準

FFP の主な投与目的は凝固因子の補充である. したがって, 凝固因子がどのくらい不足しているのかを PT や APTT 検査で投与前に確認しておくのが原則であり, その結果と出血症状をもとに FFP の適応を決定する(表 2). 凝固検査異常はあっても出血症状のない患者には FFP を予防的には投与しない. FFP 投与に関する質の高い大規模無作為試験はまだ行われていないので確立された投与基準はないことになるが, 現時点で最も確からしいエビデンスは, これまでの小規模無作為比較試験がいかなる患者を対象にしても FFP を予防的に投与する有用性を否定し続けていることである.

2. FFP の投与量

止血効果が期待できる凝固因子活性は正常値の 20～30% 以上とされている. このレベルの上昇に必要な FFP 投与量は約 10 ml/kg と計算される(表 3). しかし, ほとんどの患者で凝固因子の産生低下や消費亢進などの病態が存在するため, 理論通りには上昇しない. 実際に凝固異常のある ICU 患者 10 名を対象に平均 12.2 ml/kg の FFP を投与したときの各凝固因子活性の上昇は 10% 程度にとどまっており, 30 ml/kg の投与で 30～40% の上昇が得られたことから[4], 実際の患者では少なくとも 20 ml/kg 程度の FFP が必要と推測される. これは体重 50 kg の患者で約 1,000 ml の FFP に相当し,

■ 肝硬変でPTの正常化を目指してFFPを投与すると循環過負荷による病態悪化を招く.
■ DICで止血を図る必要のある場合は抗凝固薬とFFPの併用が必須である.
■ 大量出血ではFFPとフィブリノゲン製剤の併用が補完的に作用する.

表3 FFP輸注時の凝固因子活性上昇予測値(%)

体重(kg)		5	10	15	20	25	30	35	40	45	50	60	70	80	90
FFP投与単位	2単位	96	48	32	24	19	16	14	12	11	10	8	7	6	5
	4単位		96	64	48	38	32	27	24	21	19	16	14	12	11
	6単位			96	72	58	48	41	36	32	29	24	21	18	16
	8単位				96	77	64	55	48	43	38	32	27	24	21
	10単位					96	80	69	60	53	48	40	34	30	27

FFP 2単位はFFP-LR240製剤1本(240 m*l*)に相当.
補充凝固因子の血中回収率を80%として算出.
凝固因子上昇値(%)=FFP投与量(m*l*)×80(%:回収率)/[体重(kg)×40(m*l*/kg):循環血漿量]
(日本輸血・細胞治療学会・テルモ株式会社編:輸血療法マニュアル 改訂第5版, 2013.5の表より改変引用)

このような大量のFFP投与はNa負荷と循環血液量増加による心不全を惹起しかねない.後天性凝固異常では10%程度の凝固因子活性は残存していることも多く,大量出血例を除けば表3に示した投与量程度にとどめる方が安全と思われる.

3. 肝障害

凝固因子は肝臓で合成されるため,肝障害により複数の凝固因子活性が低下する.それを反映する検査としてプロトロンビン時間prothrombin time(PT)がよく使われており,止血に必要なレベルである20~30%の凝固因子が存在する場合,PT-INRはほぼ1.7になる.PT-INR 2.0の患者では500 m*l*のFFP投与でPT-INR 1.7まで改善するが,PT-INR 3.0, 4.0の患者ではそれぞれ1,500 m*l*, 2,000 m*l*のFFPを必要とする[5].非代償性肝硬変でPT-INRが延長している患者の観血的処置前にFFPをそのくらい投与するとPT-INRの改善は得られるかもしれないが,それで出血が少なくなることをきちんと証明したスタディはなく,逆に循環過負荷による腹水の増悪や心不全の発症が懸念される.

4. disseminated intravascular coagulation syndrome(DIC)

DICは基礎疾患が治癒すると自然に改善する病態なので,基礎疾患の治療が原則である.DICでは微小血栓の形成に凝固因子が使われて減少しているので単にFFPを投与しても血栓の増悪をきたす.したがって,抗凝固薬によって凝固因子の消費を抑制したうえでFFPを輸注するのが基本である.合成プロテアーゼインヒビター(FOY,フサン®),トロンボモジュリン製剤(リコモジュリン®)やアンチトロンビン製剤はDICの治療でよく用いられる薬剤だがすべて抗凝固薬であり元来止血作用はない.活動性出血を直ちに止めなければならない病態ではFFPや血小板の併用が必須になる.

5. 大量出血

大量出血は血漿中の凝固因子の喪失をもたらし,大量の補液と赤血球輸血によって血漿が希釈されている状態になることから希釈性凝固障

■ FFP の治療効果は投与前後の凝固検査と出血症状の改善程度で評価する.

■ 輸血製剤の効果が不十分なときは局所処置や抗線溶薬などを考慮する.

害と呼ばれる病態を生じる. 希釈性凝固障害は凝固因子欠乏によるトロンビン産生障害とフィブリノゲン欠乏によるフィブリン網形成障害の二つの機序の複合からなっている. FFP は両機序の改善作用を有するが大量投与を必要とするため時間がかかるという欠点がある. 数分で強固なフィブリン網を形成させて止血を図るにはフィブリノゲン製剤が適しているが, トロンビン産生作用はないので FFP の併用が必要となる.

6. FFP の治療効果判定

FFP 輸注は凝固因子補充療法であるので, 輸注後に凝固検査を行って効果を評価するのが原則となる. しかし, 上述したように検査値の正常化を目指して FFP を投与すると大量の FFP を要する事態となり心不全を起こしかねない. 投与前後での検査値と出血症状の改善の程度を勘案して有効性を評価する. 凝固検査を行わず漫然と FFP を投与し続けることは慎むべきである.

おわりに

輸血療法無効時には

血小板減少や血液凝固異常のある患者に適切な輸血製剤を投与しても出血をコントロールできないことはよくある. この最大の原因は, 出血局所の組織破綻にある. 例えば, IVH カテーテル挿入部位から滲み出る出血(oozing)が持続する患者では, 局所へのトロンビン散布や縫合の追加などの局所処置が, FFP の全身投与よりもはるかに有効である. oozing 部位以外に紫斑などの全身出血症状がない患者ではなおさらである. また, 口腔内の出血は線溶の関与がきわめて大きいので, 抗線溶薬の併用がしばしば奏効するなど, 別機序の止血アプローチも重要である. 出血要因を症例ごとに検索し, 血小板・FFP にこだわらない止血処置を考えていく必要がある.

文 献

1) 羽藤高明：血小板輸血トリガー値の検証. 日輸血細胞治療会誌 57：436-441, 2011
2) Wandt, H. et al.：Therapeutic platelet transfusion versus prophylactic transfusion in patients with hematological malignancies：an open-label, multicentre, randomized study. Lancet 380：1309-1416, 2012
3) Stanworth, S.J. et al.：A no-prohylaxis platelet-transfusion strategy for hematologic cancers. N Engl J Med 368：1771-1780, 2013
4) Chowdhury, P. et al.：Efficacy of standard dose and 30 ml/kg fresh frozen plasma in correcting laboratory parameters of haemostasis in critically ill patients. Br J Haematol 125：69-73, 2004
5) Holland, L.L. et al.：Toward rational fresh frozen plasma transfusion. The effect of plasma transfusion on coagulation test results. Am J Clin Pathol 126：133-139, 2006

Clinical Case Reports

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CASE REPORT

STEC:O111-HUS complicated by acute encephalopathy in a young girl was successfully treated with a set of hemodiafiltration, steroid pulse, and soluble thrombomodulin under plasma exchange

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Key Clinical Message

We report a 14-year-old girl, who developed shigatoxin-producing *E. coli* (STEC)-HUS complicated by encephalopathy. She was successfully treated with hemodiafiltration, high-dose methylprednisolone pulse therapy, and soluble recombinant thrombomodulin under plasma exchange. von Willebrand factor multimers analysis provides potential insights into how the administered therapies might facilitate successful treatment of STEC-HUS.

Keywords

Encephalopathy, *Escherichia coli* O111, hemolytic uremic syndrome, plasma exchange, recombinant soluble thrombomodulin, von Willebrand factor.

Introduction

Hemolytic uremic syndrome (HUS) is a life-threatening disease, characterized by microangiopathic hemolytic anemia, destructive thrombocytopenia, and renal failure [1]. Most HUS occurs in association with Shiga toxin-producing *Escherichia coli* (STEC) infection [2]. Patients with STEC-HUS generally recover with fluid therapy and hemodialysis. Mortality is high among STEC-HUS patients with encephalopathy, despite treatments including plasma exchange, steroid pulse, and more recently eculizumab [3]. In recent STEC outbreaks in the United States (STEC-O111) and Germany (STEC-O104) in 2008 and 2011, respectively [4, 5], STEC-HUS incidence and mortality were 16.7% and 3.8% and 22% and 3.7%, respectively.

In 2011, an outbreak of STEC-O111 and/or -O157 infection in Toyama, Japan occurred following raw meat ingestion in a barbecue restaurant chain. Overall, 181 patients were infected, of whom 34 developed STEC-HUS (18.8%) including 21 with encephalopathy (61.8%) and five deaths (14.7%; all with encephalopathy) [6–8]. Ten STEC-HUS patients were aged 1–14 years, including eight with encephalopathy [7]. Seven children including five

with encephalopathy recovered and three died [7]. We report clinical and laboratory findings for a 14-year-old girl in the Toyama series with STEC-HUS and encephalopathy.

Case Report

In April 2011, a 14-year-old girl ingested raw meat in a barbecue restaurant in Toyama, and then traveled to Osaka. Bloody diarrhea developed 5 days later. At a local hospital, levofloxacin was prescribed without improvement. Six days later after raw meat ingestion, she was transferred to Yodogawa Christian hospital. Almost simultaneously, multiple outbreaks of hemorrhagic enterocolitis due to STEC: O111 (producing both shiga-toxin-1 and -2) were reported from several hospitals around Toyama. All affected patients had eaten raw meats in the same chain restaurants around Toyama. Admission laboratory findings included: white blood cell (WBC) [24,700/μl], red blood cell (RBC) [5.28 $\times$ 10^6/μL], hemoglobin (Hb) [16.7 g/dL], platelet [143 $\times$ 10^3/μL], C-reactive protein (CRP) [3.55 mg/dl], lactate dehydrogenase (LDH) [227 IU/L], blood urea nitrogen (BUN) [15.6 mg/dL], creatinine (Cr) [0.69 mg/dL], normal hemostatic tests, proteinuria, and no hematuria. Stool cultures showed normal flora, stool shigatoxin stool was negative, and both the antigens of STEC:O111 and O157 in stool were negative.

On day 3, the patient developed anemia (RBC [2.63 $\times$ 10^6/μL], Hb [8.2 g/dL], LDH [1148 IU/L], haptoglobin [8 mg/dl], and thrombocytopenia [12,000/μl], with an increase in BUN [26.6 mg/dL] and Cr [1.06 mg/dL] as shown in Figure 1). Schistocytes were seen in the peripheral blood smear. Plasma ADAMTS13 activity levels were 43% of normal. The patient became anuric and comatose (Glasgow Coma Scale [GCS] 14). Continuous hemodiafiltration was initiated with plasma exchange. On day 5, pleural effusions developed, respiratory function worsened, and consciousness deteriorated further. Intubation was performed. Brain magnetic resonance imaging showed high intensity areas in the bilateral thalamus and basal ganglia, and part of the pontine tegmentum on T2 FLAIR images (Fig. 1 Inset). Acute encephalopathy developed. STEC-HUS was diagnosed. High-dose methylprednisolone pulse therapy [500 mg/day] for days 5–7 was administered. On day 6, serum antibodies to STEC:O111 antigen were noted. On day 9, hemolysis worsened, whereas severe thrombocytopenia persisted. Plasma exchange was increased to twice daily. A second 3-day course of a high-dose methylprednisolone pulse therapy was administered. Gabexate mesilate, a synthetic anticoagulant was administered. Serum levels of fibrin/fibrinogen degradation product (FDP) and thrombin–antithrombin complex (TAT) increased to 120 μg/mL and 24.3 ng/mL, respectively. Soluble recombinant thrombomodulin (130 units/kg/day) was infused during days 9–14. Clinical and laboratory findings subsequently improved, including thrombocytopenia, hemolysis, and renal function (Fig. 1). Extubation occurred on day 22. Plasma exchange was tapered, and discontinued on day 24. After rehabilitation, the patient was discharged without appreciable sequelae on day 64.

Retrospective analyses of stored plasma samples were performed. Plasma samples from admission showed that levels of the following cytokines were not elevated: interleukin (IL)-6 [4 pg/mL (normal: <4)], IL-8 [59 pg/mL (normal: <2)], and tumor necrosis factor (TNF)α [12 pg/mL (normal: <15)]. In contrast, plasma samples from admission identified elevated levels of neopterin [98 nmol/L (normal: <5)], soluble form TNF receptor type I (sTNF-RI) [13,200 pg/mL (normal: 484–1407)], sTNF-RII [18,300 pg/mL (normal: 829–2262)], and tau protein [344 pg/mL (normal: undetectable)]. Plasma samples from day 3 identified reduced plasma ADAMTS13 activity (43%) levels and high levels of plasma VWF antigen levels (605% of normal).

Retrospective analysis of plasma VWF multimer patterns using citrated plasma samples (frozen at -80°C) was also performed (Fig. 2). During the acute phase, no high-to-intermediate sized VWF multimers were identified in samples taken three and 13 days prior to initiation of plasma exchange. After each plasma exchange, VWF multimer patterns were present, although high-sized VWF multimers continued to be absent. Plamsa exchange was performed once or twice daily until day 20, then tapered, and discontinued on day 24. UL-VWF multimers appeared in plasma at days 21 and 24, and disappeared at day 61 just before discharge. At discharge, plasma levels of VWF and ADAMTS13 had returned to almost normal ranges.

Discussion

We report a patient with STEC-HUS, mild-to-moderate reduction of plasma ADAMTS13 activity, and increased plasma levels of VWF antigen. Despite persistent thrombocytopenia in the acute phase, VWF multimers were degraded on one occasion and highly multimerized on a different occasion. Therapy with continuous hemodiafiltration, high-dose methylprednisolone pulse therapy and soluble recombinant thrombomodulin was successful and the patient was discharged without any deficits. In explaining our findings, several factors should be considered.

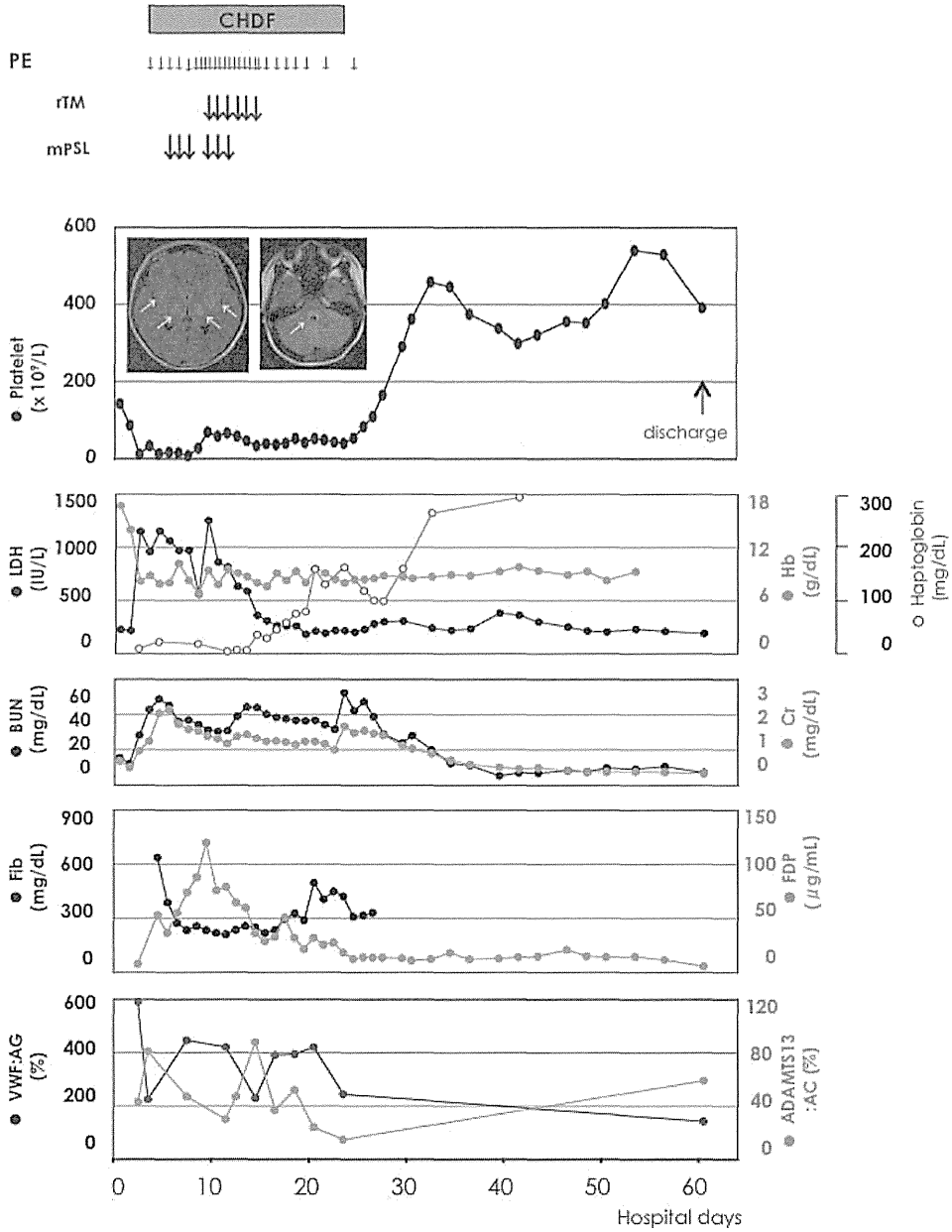

Figure 1. Clinical course in a 14-year-old girl with STEC-HUS complicated by acute encephalopathy after admission.

First, identification of UL-VWF multimers in this patient differs from the VWF pattern usually seen with STEC-HUS where the multimers are usually depleted. UL-VWFMs, stored in Weibel–Palade bodies (WPBs) of vascular endothelial cells, are released upon stimulation by inflammatory cytokines, such as IL-6, IL-8, and TNFα [9]. Likewise, UL-VWFMs are released into the circulation by injured vascular endothelial cells. On admission, plasma levels of cytokines including IL-8, neopterin, TNF-RI and RII, and tau protein were high, indicating vascular injury, inflammation, and neurological cell damages [6]. Also, the B-subunit of shigatoxin-1 and -2, both AB5-holotoxins, binds to globotrialosyl ceramide (Gb3) by which UL-VWFMs are released from Weibel–Palade bodies [10]. Shigatoxin binds to Gb3, internalizes, and blocks protein synthesis by attachment to ribosomal RNA. Shigatoxin also directly enhances platelet aggregation under high and low shear stress at very low concentrations [11]. Thus, in our patient, UL-VWFM, may have been released excessively from activated vascular endothelial cells, was involved in platelet thrombi formation, and then was consumed by proteases released from platelets and/or leucocytes.

Second, our findings may explain how plasma exchange may have had therapeutic benefit in this patient. In par-

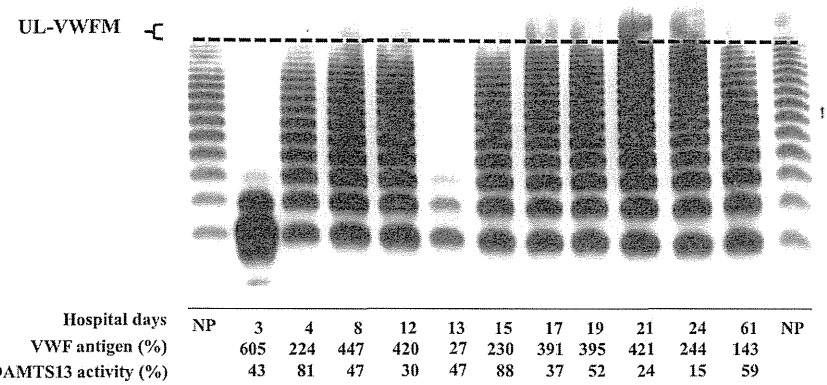

Figure 2. Change of VWF multimer patterns during the acute phase.

ticular, plasma exchange might work bifunctionally: one effect was to reduce concentrations of various cytokines, UL-VWFM, and shigatoxin, and the other effect was to supply normal VWFM (for hemostasis). During the acute phase of STEC-HUS, the STEC vigorously produces shigatoxin, which consistently activates platelets, even at low concentrations (pg/ml). So, plasma exchange alone for STEC-HUS is likely to be inefficient, unless shigatoxin function is blocked. Hence, in addition to basic supportive therapy for STEC-HUS such as dialysis and fluid therapy, cytokine adsorption is favorable, and high-dose methylprednisolone pulse therapy might suppress cytokine production [12].

Third, in comparison to previous reports, the occurrence of acute encephalopathy associated with STEC-HUS in Toyama was high, and the deceased cases had encephalopathy. This toxicity is attributable to brain edema, presumably due to increased vascular permeability and/or severe vascular endothelial cell injuries mediated by shigatoxin itself and cytokines, yet the mechanism is not fully understood [13]. Strains of STEC:O111 isolated in Toyama predominantly produced shigatoxin-2, which is more toxic than shigatoxin-1. However, a peculiar MRI finding on high intensity areas, often symmetrical in thalamus, basal ganglia, and pontine tegmentum, has not been favorably addressed [14].

Fourth, common therapeutic features on seven survived childhood patients in Toyama included continuous hemodiafiltration, high-dose methylprednisolone pulse therapy, and recombinant thrombomodulin. High-dose intravenous immunoglobulin infusion was administered to six of the seven survivors. Administration of recombinant thrombomodulin may have been particularly important, as this drug has been available in Japan as treatment for disseminated intravascular coagulation (DIC) since 2008 [15]. Recombinant thrombomodulin is a multifunctional protein. A lectin-like domain directly absorbs and neutralizes high mobility group box1 (HMGB1), which is a pro-inflammatory cytokine that acts as a lethality factor when endotoxin shock occurs [16]. Also, EGF-like domains 4–6 of the recombinant thrombomodulin can bind thrombin and inactive the catalytic activity of thrombin. The thombin–recombinant thrombomodulin complex can accelerate activation of protein C and thrombin activatable fibrinolytic inhibitor (TAFI) to activated protein C and TAFIa, respectively. In turn, activated protein C generates anticoagulant action via inactivation of Va and VIIIa and TAFIa suppresses complement activation via inactivation of C3a and C5a [15]. As the action of recombinant thrombomodulin on platelets remains unclear, we are unable to directly address how recombinant thrombomodulin can resolve STEC-HUS. There are at least two possibilities: one is direct inhibitory activity to platelet aggregation, and the second is to block fibrin clot formation over platelet thrombi, as suggested by significant increases of FDP and TAT during the clinical course before recombinant thrombomodulin is administered.

In conclusion, we report a novel therapy for STEC-HUS. VWF-dependent hemostatic defect that is generated in STEC-HUS appears to have been restored by plasma exchange. Hypercoagulability, presumably induced by shigatoxin or cytokine storms, appears to have been suppressed with high-dose methylprednisolone pulse therapy and recombinant thromobomodulin.

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Conflict of Interest

Nothing to declare.

References

1. Ruggenenti, P., M. Noris, and G. Remuzzi. 2001. Thrombotic microangiopathy, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura. Kidney Int. 60:831–846.
2. Noris, M., and G. Remuzzi. 2009. Atypical hemolytic-uremic syndrome. N. Engl. J. Med. 361:1676–1687.
3. Nathanson, S., T. Kwon, M. Elmaleh, M. Charbit, E. A. Launay, J. Harambat, et al. 2010. Acute neurological involvement in diarrhea-associated hemolytic uremic syndrome. Clin. J. Am. Soc. Nephrol. 5:1218–1228.
4. Frank, C., D. Werber, J. P. Cramer, M. Askar, M. Faber, M. an der Heiden, et al. Epidemic profile of Shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany. The New England journal of medicine 2011. 365:1771–1780.
5. Piercefield, E. W., K. K. Bradley, R. L. Coffman, and S. M. Mallonee. 2010. Hemolytic uremic syndrome after an *Escherichia coli* O111 outbreak. Arch. Intern. Med. 170:1656–1663.
6. Shimizu, M., M. Kuroda, N. Sakashita, M. Konishi, H. Kaneda, N. Igarashi, et al. 2012. Cytokine profiles of patients with enterohemorrhagic *Escherichia coli* O111-induced hemolytic-uremic syndrome. Cytokine 60:694–700.
7. Taneichi, H., M. Konishi, N. Igarashi, H. Kaneda, H. Matsukura, K. Ogura, et al. 2013. An outbreak of enterohemorrhagic *Escherichia coli* O111 associated with the consumption of raw beef in Toyama in Japan. J. Jpn Pediatr. Soc. 117:1409–1415.
8. Takanashi, J., H. Taneichi, T. Misaki, Y. Yahata, A. Okumura, Y. Ishida, et al. 2014. Clinical and radiologic features of encephalopathy during 2011 *E. coli* O111 outbreak in Japan. Neurology 82:564–572.
9. Bernard, G. R., J. L. Vincent, P. F. Laterre, S. P. LaRosa, J. F. Dhainaut, A. Lopez-Rodriguez, et al. 2001. Recombinant human protein CWEiSSsg. Efficacy and safety of recombinant human activated protein C for severe sepsis. N. Engl. J. Med. 344:699–709.
10. Huang, J., D. G. Motto, D. R. Bundle, and J. E. Sadler. 2010. Shiga toxin B subunits induce VWF secretion by human endothelial cells and thrombotic microangiopathy in ADAMTS13-deficient mice. Blood 116:3653–3659.
11. Yagi, H., N. Narita, M. Matsumoto, Y. Sakurai, H. Ikari, A. Yoshioka, et al. 2001. Enhanced low shear stress induced platelet aggregation by Shiga-like toxin 1 purified from *Escherichia coli* O157. Am. J. Hematol. 66:105–115.
12. Fujii, J., Y. Kinoshita, A. Matsukawa, S. Y. Villanueva, T. Yutsudo, and S. Yoshida. 2009. Successful steroid pulse therapy for brain lesion caused by Shiga toxin 2 in rabbits. Microb. Pathog. 46:179–184.
13. Greinacher, A., S. Friesecke, P. Abel, A. Dressel, S. Stracke, M. Fiene, et al. 2011. Treatment of severe neurological deficits with IgG depletion through immunoadsorption in patients with *Escherichia coli* O104:H4-associated haemolytic uraemic syndrome: a prospective trial. Lancet 378:1166–1173.
14. Lobel, U., B. Eckert, O. Simova, M. Meier-Cillien, S. Kluge, C. Gerloff, et al. 2014. Cerebral magnetic resonance imaging findings in adults with haemolytic uraemic syndrome following an infection with *Escherichia coli*, subtype O104:H4. Clin. Neuroradiol. 24:111–119.
15. Saito, H., I. Maruyama, S. Shimazaki, Y. Yamamoto, N. Aikawa, R. Ohno, et al. 2007. Efficacy and safety of recombinant human soluble thrombomodulin (ART-123) in disseminated intravascular coagulation: results of a phase III, randomized, double-blind clinical trial. J. Thromb. Haemost. 5:31–41.
16. Hagiwara, S., H. Iwasaka, K. Goto, Y. Ochi, S. Mizunaga, T. Saikawa, et al. 2010. Recombinant thrombomodulin prevents heatstroke by inhibition of high-mobility group box 1 protein in sera of rats. Shock 34:402–406.

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Case Report

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A Unique Case Involving a Female Patient with Upshaw-Schulman Syndrome: Low Titers of Antibodies against ADAMTS13 prior to Pregnancy Disappeared after Successful Delivery

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Keywords
Upshaw-Schulman syndrome · Pregnancy · ADAMTS13 antibody · *ADAMTS13* gene mutation · Fresh frozen plasma

Summary
Background: Upshaw-Schulman syndrome (USS) is usually suspected based on severe deficiency of ADAMTS13 activity without ADAMTS13 antibody, but the definitive diagnosis is made by *ADAMTS13* gene analysis. We present a unique case of USS with low titers of ADAMTS13 antibodies before pregnancy. Interestingly, titers of ADAMTS13 antibodies decreased to almost undetectable levels after delivery. **Case Report**: In patient LL4, the diagnosis of USS was confirmed at age 27 by *ADAMTS13* gene analysis. She became pregnant at age 30. During the pregnancy, she received regular fresh frozen plasma (FFP) infusion. Plasma von Willebrand factor levels increase as pregnancy progresses. To prevent platelet thrombi, much more ADAMTS13 supplementation is necessary during late gestation in patients with USS. Therefore, we shortened the interval between and increased the volume of FFP infusions as pregnancy progressed. At 39 weeks, she delivered a healthy baby girl. Before pregnancy, she had low titers of both neutralizing and binding anti-ADAMTS13 antibodies. Despite frequent FFP infusions, titers of the antibodies did not increase, but rather decreased to almost undetectable levels during pregnancy. **Conclusion**: Both the neutralizing and binding antibodies against ADAMTS13 decreased to almost undetectable levels after delivery in this patient, which can be caused by an immunological reset.

Introduction

Upshaw-Schulman syndrome (USS) is caused by a deficiency of ADAMTS13 activity due to a mutation in its gene [1]. ADAMTS13 specifically cleaves unusually large von Willebrand factor (VWF) multimers (UL-VWFMs) released from vascular endothelial cells. When ADAMTS13 activity is deficient, UL-VWFMs are not cleaved, which induces platelet thrombi formation in the microcirculation under high shear stress. Deficiency of ADAMTS13 activity is also caused by autoantibodies against ADAMTS13 in patients with acquired thrombotic thrombocytopenic purpura (TTP) [2]. There are two types of ADAMTS13 autoantibodies. One type acts as an inhibitor of ADAMTS13 function, and the other type binds to ADAMTS13, accelerating its clearance from the circulation. USS is usually suspected to be based on severe deficiency of ADAMTS13 activity without the presence of autoantibodies, but the definitive diagnosis is usually made by *ADAMTS13* gene analysis.

USS patients often experience episodes of severe neonatal jaundice with a negative Coombs test requiring an exchange blood transfusion as well as repeated episodes of thrombocytopenia and

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