Introduction

ABO-incompatible transfusion preceded Landsteiner's discovery of human blood groups, but persists more than 100 years later as an important cause of adverse events due to human error [1–3]. Haemovigilance systems in Europe and North America target ABO-incompatible blood transfusion [1,2,4]. In Japan, Red Cross blood centres collect haemovigilance data, but specifically target transfusion-transmitted virus infections and immune phenomena such as allergic reactions, transfusion-related acute lung injury, and transfusion-associated graft-vs.-host disease [5]. Therefore, the actual incidence of ABO-incompatible blood transfusion in our country has been uncertain. In order to investigate and guide methods of prevention, consecutive national surveys were initiated by the Japanese Society of Blood Transfusion (now the Japanese Society of Transfusion Medicine and Cell Therapy) [6,7].

Materials and methods

The Japan Society of Blood Transfusion developed anonymous questionnaires, targeting 777 hospitals from January 1995 to December 1999, and 1355 hospitals from January 2000 to December 2004. Data were analysed and reported in 2000 and in 2005. The first survey solicited cases arising from whole blood (WB), red cell concentrate (RCC) and fresh frozen plasma (FFP) transfusions at 777 hospitals, each having at least 300 beds. The scope of the second survey expanded to include cases arising from platelet concentrate transfusions, and targeted 1355 hospitals, including 777 of the same hospitals targeted in the first survey and 578 additional hospitals with fewer than 300 beds, where at least one transfusion specialist was working. Not only accidents but also incidents (errors without adverse reactions) were solicited. In regard to transfusion oversight, blood transfusion management systems and laboratory testing outside of core hours were investigated in first survey (Tables 1 and 2). To these, the second survey added utilization of electronic equipment for blood transfusion management and product testing (Tables 3 and 4).

Table 1 ABO-incompatible blood transfusion questionnaire form 1 of the first survey (1 January 1995 to 31 December 1999)

I. Did the ABO-incompatible blood transfusion occur in the past 5 years (1 January 1995 to 31 December 1999)?
(The targets are whole blood, red cell concentrates, and fresh frozen plasma; and platelets concentrates should be excluded.)
(1) Yes (Please give details using investigation form 2 on the next page.)
(2) No

II. Questions on system of blood transfusion management

1. Number of hospital beds: Select from the following:
(1) 300 to less than 400 beds
(2) 400 to less than 500 beds
(3) 500 to less than 600 beds
(4) 600 to less than 700 beds
(5) 700 to less than 800 beds
(6) 800 to less than 900 beds
(7) 900 to less than 1000 beds
(8) More than 1000 beds
2. Amount of transfused blood components during the last fiscal year: Select from the following:
(1) 3000 to less than 10 000 units
(2) 10 000 to less than 20 000 units
(3) 20 000 to less than 30 000 units
(4) 30 000 to less than 40 000 units
(5) 40 000 to less than 50 000 units
(6) More than 50 000 units
3. Section that manages blood supply:
(1) Blood transfusion service
(2) Laboratory
(3) Pharmacy
(4) Others
4. Pretransfusion testing out of core hours:
(1) Duty by laboratory technician
(2) The doctor takes charge
(3) Laboratory technician's system of on call
(4) Others
5. Doctor accredited by the Japan Society of Blood Transfusion:
(1) Yes
(2) No
6. Laboratory specialist accredited by the Japan Society of Blood Transfusion:
(1) Yes
(2) No
7. Hospital transfusion therapy committee:
(1) Yes
(2) No
8. Please describe any special method to prevent of ABO-incompatible blood transfusion in your hospital.

Results

A 74·4% response rate was achieved in the 1995–99 survey, corresponding to 578 of 777 hospitals. A 61·2% response rate was achieved in the 2000–04 survey, corresponding to 829 of 1355 hospitals. From 578 participating hospitals in the first survey came 166 case reports, vs. only 60 case reports from the 829 hospitals participating in the second survey including six cases reported from hospitals with fewer 300 beds (Table 5). These cases include those without adverse reactions. Nevertheless, the number of fatalities reported in each survey was nearly equal: nine in the first survey and eight in the second. In the second survey, the mean number of transfused blood components reported from 540 hospitals during survey period was 14 855 bags, but in first survey the exact number of transfused blood components was not

Table 2 ABO-incompatible blood transfusion questionnaires form 2 (case report) of the first survey (1 January 1995 to 31 December 1999)

1. Content of case:

(Please describe details and the reason for the discovery of ABO-incompatible blood transfusion.)

2. Persons concerned who made a mistake:
   (1) Doctor
   (2) Nurse
   (3) Laboratory technician
   (4) Others ( )
3. Time period:
   (1) Regular (daylight) hours
   (2) Out of core hours
4. Was it an urgent blood transfusion?
   (1) Yes
   (2) No
5. Site of blood transfusion:
   (1) Ward
   (2) Operation room
   (3) ICU
   (4) Emergency room
   (5) Others
6. Blood product:
   (1) Whole blood
   (2) Red cell concentrates
   (3) Fresh frozen plasma
7. ABO type:
   Blood type of blood preparation
   Patient's blood type
8. Amount of blood transfusion (ml):
9. How long did it take you to become aware of ABO-incompatible blood transfusion from the beginning of transfusion?
10. Did you explain the situation to the patient and family?
    (1) Yes
    (2) No
    (3) Uncertain
11. Was there any symptom of shock?
    (1) Yes
    (2) No
    (3) Unknown
12. Was there any sign of haemolysis?
    (1) Yes
    (2) No
    (3) Unknown
13. Was there any sign of disseminated intravascular coagulation?
    (1) Yes
    (2) No
    (3) Unknown
14. Was there any sign of renal insufficiency?
    (1) Yes
    (2) No
    (3) Unknown
15. What kind of treatment was performed?
16. Outcome:
    (1) Death
    (2) Survival with adverse effects
    (3) Survival without adverse effects
17. Improvement plan concerning ABO-incompatible blood transfusion prevention adopted after the case occurred:
18. Others

(If you think there is anything else pertinent to this case, please describe the details.)

Table 3 ABO-incompatible blood transfusion questionnaire form 1 of the second survey (1 January 2000 to 31 December 2004)

I. Did the ABO-incompatible blood transfusion occur in the past 5 years (1 January 2000 to 31 December 2004)?
(The targets are whole blood, red cell concentrates, fresh frozen plasma, and platelet concentrates.)
(1) Yes (Please give details using investigation form 2.)
(2) No

II. Questions on system of blood transfusion management

1. How many beds does your hospital have?
( ) beds

2. How many units of total blood transfusion products were administered over 5 years 1 January 2000 to 31 December 2004?

Whole blood	( ) units, ( ) bags
Red cell concentrates	( ) units, ( ) bags
Fresh frozen plasma	( ) units, ( ) bags
Platelets concentrates	( ) units, ( ) bags

3–8. Same as those of the first survey

9. Do you electronically verify patients and blood products before transfusion at bedside?
(1) Yes
(2) No
(3) Only in a part of the ward

10. Is a computer-based ordering system used to request the blood supply?
(1) Yes
(2) No
(3) Its introduction is scheduled

11. Is the ordering computer system used to request the pretransfusion testing?
(1) Yes
(2) No
(3) Its introduction is scheduled

12. Is a computer-based system used for the stock-taking and managing the delivery of the blood products?
(1) Yes
(2) No
(3) Its introduction is scheduled

13. Is an automatic blood transfusion testing machine used?
(1) Yes
(2) No
(3) Its introduction is scheduled

Table 4 ABO-incompatible blood transfusion questionnaire form 2 (case report) of the second survey (1 January 2000 to 31 December 2004)

1–18. Same as those of the first survey

19. Did it occur before introducing the portable digital assistant to blood transfusion confirmation at the bed side?
(1) Yes
(2) No

Table 5 Analysed data

	First survey	Second survey	
Survey period	1 January 1995 to 31 December 1999	1 January 2000 to 31 December 2004	
Target hospital	777	1355	
> 300 beds	777	777[a]	
< 300 beds	0	578	
Response (%)	578 (74·4)	829 (61·2)	
> 300 beds	578 (74·4)	502 (64·2)	
< 300 beds		327 (55·7)	
Reported cases[b]			
	WB + RCC + FFP[c]	RCC + FFP[d]	PC[e]
> 300 beds	166	48	6
< 300 beds	0	4	2
Total	166	52	8

[a]777 hospitals the same as those targeted in the first survey.
[b]Reported cases including those without adverse reactions.
[c]Cases arising from whole blood (WB), red cell concentrate (RCC), and fresh frozen plasma (FFP), including those arising from unknown components.
[d]Cases arising from RCC and FFP, including those arising from unknown components.
[e]Cases arising from platelet concentrate.

Table 6 Number of reports according to the type of blood product

	First survey[a]	Second survey[b]
Whole blood major mismatch	3	0
Whole blood minor mismatch	2	0
Red cell concentrate major mismatch	48	22
Red cell concentrate minor mismatch	38	9
Fresh frozen plasma	71	19
Platelet concentrate	Not reported	8
Unknown	4	2
Total	166	60

[a]1 January 1995 to 31 December 1999.
[b]1 January 2000 to 31 December 2004.

collected. The number of reported cases of ABO-incompatible blood transfusion according to the number of hospital beds is shown in Fig. 1. A decrease in the number of reported cases was recognized in large hospitals, defined as having more than 700 beds. Table 6 shows the numbers of reported cases according to the type of blood product. A decrease of RCC minor mismatch and FFP was more remarkable than that of RCC major mismatch. Outcomes in patients receiving RCC major mismatch included nine deaths in the first survey and eight in the second. The cause of death includes the possibility of underlying disease in nine of 17 cases according to the contents of cases in questionnaire form 2. In six of the remaining eight deaths, unambiguously due to ABO-incompatible transfusion, the patients were of group O blood type. Data from the second survey suggest a risk of ABO-incompatible

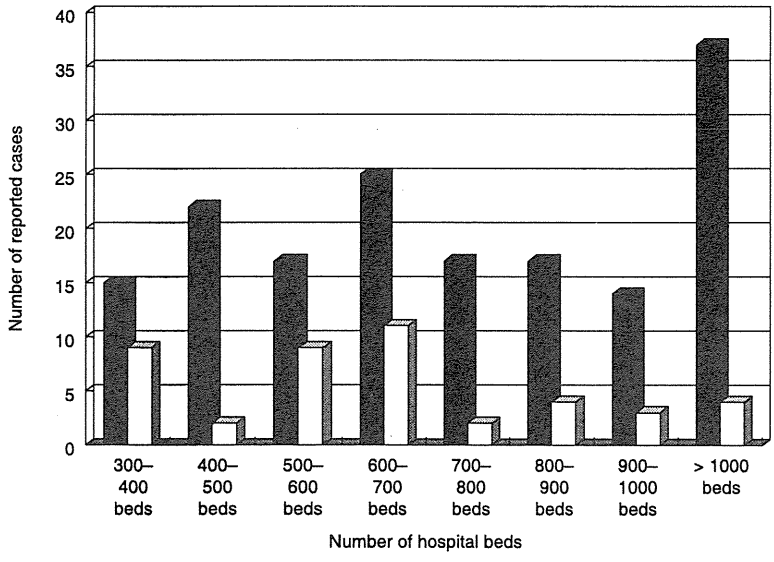

Fig. 1 Number of reported cases of accidental ABO-incompatible blood transfusion of red cell concentrate and fresh frozen plasma according to the number of hospital beds.
■: Number of reported cases of ABO-incompatible blood transfusion of whole blood, red cell concentrates, and fresh frozen plasma in the first survey (1 January 1995 to 31 December 1999).
□: Number of reported cases of ABO-incompatible blood transfusion from red cell concentrates and fresh frozen plasma reported only from hospitals having at least 300 beds in the second survey (1 January 2000 to 31 December 2004).

Table 7 Pretransfusion testing out of core hours

	Number of hospitals		
	First survey[a]		Second survey[b]
	> 300 beds (%)	> 300 beds (%)	< 300 beds (%)
Duty of laboratory specialist	347 (60·35)	476 (75·1)	26 (13·9)
Laboratory specialist on call	163 (28·35)	147 (23·2)	157 (83·9)
The doctor takes charge	43 (7·5)	4 (0·6)	2 (1·1)
Others	22 (3·8)	7 (1·1)	2 (1·1)
Total	575 (100)	634 (100)	187 (100)

[a]1 January 1995 to 31 December 1999.
[b]1 January 2000 to 31 December 2004.

transfusion as 1 : 200 000 and a risk of the death as 1 : 3 000 000. The status of pretransfusion testing out of core hours is shown in Table 7. Electronic correlation of patients and blood products seems to have had limited implementation in 1999, when the first survey was executed, but was reported in 8·8% of facilities in 2004 when the second survey was executed.

Main causes of transfusion error

Identification error between patient and blood product

The main cause of transfusion error was misidentification between patient and blood product: 55% of cases (91 of 166) in the first survey, and 45% (27 of 60) in the second (Table 8). RCC major mismatch comprised 36 cases in the first survey and 14 cases in the second survey. Among the reported cases, no technology-based identification systems were in place.

Phlebotomy error

Phlebotomy errors were reported in 2% of cases (four of 166) in the first survey, and 3% (two of 60) in the second. All phlebotomy errors were emergency situations where the blood typing and cross-matching were performed on the same specimen.

Prescription error

Prescription errors were reported in 11% of cases (19 of 166) in the first survey, and 13% (eight of 60) in the second. In these cases, blood component orders of an incorrect ABO blood group were sent to the laboratory. Fresh frozen plasma

Table 8 Main causes of transfusion error

	First survey[a]	Second survey[b]
Identification error	91	27
Phlebotomy error	4	2
Prescription error[c]	19	8
Testing error by doctor	21	10
Laboratory error outside of core hours	12	6
Laboratory error during core hours	5	4
Other	14	3
Total	166	60

[a]1 January 1995 to 31 December 1999.

[b]1 January 2000 to 31 December 2004.

[c]Blood components orders of incorrect ABO blood group.

or platelet concentrate orders of an incorrect ABO blood group sent to the laboratory were undetected by laboratory methods due to the omission of the minor cross-match. No reported prescription error was associated with an RCC major mismatch.

Testing error by doctors

Testing errors by doctors were reported in 13% of cases (21 of 166) in the first survey, and 17% (10 of 60) in the second. In hospitals where these errors arose, laboratory services for blood transfusion were not available.

Laboratory error outside of core hours

Laboratory errors outside of core hours were reported in 7% of cases (12 of 166) in the first survey, and 10% (six of 60) in the second. These errors included technical testing errors in 10 cases, issuance of the wrong units in four cases, and use of the wrong patient sample for testing in one case, and, in four cases the details of errors were not reported.

Laboratory error during regular (daylight) hours

Laboratory errors during regular (daylight) hours were reported in 3% of cases (five of 166) in the first survey, and 7% (four of 60) in the second. These errors included technical testing errors in three cases, clerical error in transcription in one case, issuance of the wrong units in two cases, and use of the wrong patient sample in three cases.

Other errors

In the first survey: a wrong blood type was displayed at the bedside in one case; 11 cases had no reports about the main cause; and in two cases, a main cause could not be clearly discerned. In the second survey, two ABO-incompatible bone marrow transplant recipients received the wrong blood, and in one other case, incompatible FFP was taken from an operating room refrigerator.

Discussion

Based on data from the second survey, the risk of ABO-incompatible transfusion and that of death is about half of those reported by Serious Hazards of Transfusion (SHOT) [1]. In Japan, at least 8000 hospitals transfuse blood, perhaps more if the smallest hospitals are counted, but this investigation focused on the hospitals responsible for about 80% of the blood products transfused in Japan. The Japanese Red Cross (JRC) is the only supplier of allogeneic blood components used in Japan. The collection of allogeneic blood by a hospital transfusion service is rare and permitted in emergency cases if the JRC has failed to supply the blood products to hospitals. The total amount of all blood components supplied by the JRC corresponded to the total amount of blood components transfused in Japan. In the fiscal year of 2004, when the second survey was done, the total amount of blood components supplied by the JRC Blood Center was 16 668 784 units, and the total amount of blood components transfused in the 829 hospitals which responded to the second survey was 7 962 317 units, with about 47·8% of blood components supplied by the blood centre.

ABO-incompatible blood transfusion arises from human error [8]. Eighty per cent of ABO-incompatible blood transfusions were reported from the clinical setting of a ward or operating room and 20% were reported from a laboratory. No reported errors were associated with blood banking procedures of the JRC. There were no mislabelling of units or, weak A or B antigens typed as O. This underscores the value of an incident reporting system that collects data from hospitals, and provides analytical feedback to each facility [9–11]. Identification errors between patients and blood products provoke most RCC major mismatch transfusions. Preventive efforts are important because these errors are eminently preventable. Many hospitals had their own transfusion procedural manual, including the final identification between patients and blood products in the clinical area. In many cases, procedural deviations occurred, including half of the hospitals that maintained their own procedures. Following the first survey, a standardized blood transfusion procedure manual emphasizing the final identification between patients and blood products was developed by the Japanese Society of Blood Transfusion, and this procedure has been widely propagated through distributing a poster showing the procedural manual by the Japanese Society of Blood Transfusion and JRC [6]. The second survey collected only about 30% as many identification errors as were reported in the first survey, even with the participation of an additional 251 hospitals. It may be that the dissemination of a standard procedure contributed to a decrease in identification error. This was the main intervention undertaken to reduce the incidence of ABO-incompatible transfusion after the first survey. However, incorrect blood recipient identification at

the patient's bedside persists as the main cause of ABO-incompatible transfusion. Education programmes may be helpful to the extent that they reach all staff involved in transfusion. This is challenging under the best of circumstances, and more so where staff turnover is high. It thus behooves us to monitor employment trends in the healthcare sector. Technological interventions also have the potential to interdict human error, provided that the technology is not bypassed for reasons of expediency or lack of understanding [12–15]. The introduction of electronic correlation of patients and blood products has progressed in large-scale hospitals. Pretransfusion testing out of core hours is another problem. In 7·5% of hospitals in the first survey, laboratory services for blood transfusion out of core hours were not available, thus forcing clinicians into the role of laboratory professionals. The number of facilities where a doctor performs pretransfusion testing outside of core hours decreased from the first survey, and the number of facilities where laboratory staff perform all testing increased. Even so, laboratory staff who do not routinely perform transfusion-related testing are likely to be more error prone than those who are devoted to the blood bank or transfusion service. These were the main differences between the two surveys.

The second national survey of ABO-incompatible blood transfusion was completed 5 years after first survey. Ideally, investigative data should be collected continuously and reported at least annually, as occurs in other countries with formal haemovigilance systems [1,2]. We aspire to blend the Japanese experience described herein with international best practices described elsewhere, with the ultimate goal of mitigating the needless morbidity and mortality arising from human error.

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References

1 Stainsby D: ABO incompatible transfusions – Experience from the UK Serious Hazards of Transfusion (SHOT) scheme Transfusions ABO incompatible. *Transfus Clin Biol* 2005; 12:385–388

2 Stainsby D, Russell J, Cohen H, Lilleyman J: Reducing adverse events in blood transfusion. *Br J Haematol* 2005; 131:8–12

3 Linden JV, Wagner K, Voytovich AE, Sheehan J: Transfusion errors in New York State. An analysis of 10 years' experience. *Transfusion* 2000; 40:1207–1213

4 Andreu G, Morel P, Forestier F, Debier J, Rebibo D, Janvier G, Herve P: Hemovigilance network in France: organization and analysis of immediate transfusion incident reports from 1994 to 1998. *Transfusion* 2002; 42:1356–1364

5 Takahashi K, Juji T, Miyamoto M, Uchida S, Akaza T, Tadokoro K, Shibata Y, Ino T, Hidano A: Analysis of risk factors for post-transfusion graft-versus-host disease in Japan. Japanese Red Cross PT-GVHD Study Group. *Lancet* 1994; 343:700–702

6 Sibata Y, Inaba R, Uchikawa M, Osada K, Kurata Y, Sakamoto H, Sagawa K, Tadokoro K, Handa M, Yoshioka H, Juji T: Results of the survey on the present state of ABO-incompatible blood transfusion in Japan. *Jap J Transfusion Med* 2000; 46:545–564

7 Fujii Y, Matsuzaki M, Miyata S, Higashitani T, Inaba S, Asai T, Hoshi Y, Inada E, Kawahara K, Takamatsu J, Takahashi K, Sagawa K: Analysis of the causes of ABO-incompatible transfusions in Japan. *Jap J Transfusion Cell Therapy* 2007; 52:112–132

8 Myhre BA, McRuer D: Human error – a significant cause of transfusion mortality. *Transfusion* 2000; 40:879–885

9 Callum JL, Kaplan HS, Merkley LL, Pinkerton PH, Rabin Fastman B, Romans RA, Coovadia AS, Reis MD: Reporting of near-miss events for transfusion medicine: improving transfusion safety. *Transfusion* 2001; 41:1204–1211

10 Ibojie J, Urbaniak SJ: Comparing near misses with actual mis-transfusion events: a more accurate reflection of transfusion errors. *Br J Haematol* 2000; 108:458–460

11 Galloway M, Woods R, Whitehead S, Gedling P: Providing feedback to users on unacceptable practice in the delivery of a hospital transfusion service – a pilot study. *Transfus Med* 2002; 12:129–132

12 Pagliaro1 P, Rebulla P: Transfusion recipient identification. *Vox Sang* 2006; 91:97–111

13 Miyata S, Kawai T, Yamamoto S, Takada M, Iwatani Y, Uchida O, Imanaka H, Sase K, Yagihara T, Kuro M: Network computer-assisted transfusion – management system for accurate blood component-recipient identification at the bedside. *Transfusion* 2004; 44:364–372

14 Murphy MF, Casbard AC, Ballard S, Shulman IA, Heddle N, Aubuchon JP, Wendel S, Thomson A, Hervig T, Downes K, Carey PM, Dzik WH: Prevention of bedside errors in transfusion medicine (PROBE-TM) study: a cluster-randomized, matched-paired clinical areas trial of a simple intervention to reduce errors in the pretransfusion bedside check. *Transfusion* 2007; 47:771–780

15 Sandler SG, Langeberg A, DeBandi L, Gibble J, Wilson C, Feldman CL: Radiofrequency identification technology can standardize and document blood collections and transfusions. *Transfusion* 2007; 47:763–770

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LETTER TO THE EDITOR

Stress-induced PAI-1 expression is suppressed by pitavastatin in vivo

Koji Yamamoto · Kyosuke Takeshita · Tetsuhito Kojima · Junki Takamatsu

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Thromboembolism, including myocardial infarction, cerebral infarction, and pulmonary embolism, is frequently induced by a variety of stressors. Indeed, mental, and physical stressors decrease fibrinolytic activity [1] and contribute to the occurrence of thrombotic complications. We have already reported that plasminogen activator inhibitor-1 (PAI-1) expression is dramatically induced by restraint (immobilization) stress, a typical physicopsychological stress [2], with maximal induction in the adipose tissue in vivo, a change contributing to the development of tissue thrombosis [3]. PAI-1 regulates fibrinolysis by inhibiting plasminogen activation and elevated levels of plasma PAI-1 are observed in a variety of thrombotic conditions. In obese humans, increased plasma PAI-1 levels correlated with the amounts of visceral fat, suggesting that adipose tissue is the primary source of PAI-1 in this condition [4]. Statins, 3-hydroxy-methylglutaryl coenzyme A reductase inhibitors, have been widely used for the prevention of cardiovascular diseases primarily with their lowering serum cholesterol levels. Statins also exert pleiotropic and beneficial effects on the coagulation and fibrinolytic systems [5], which are regarded to be independent of cholesterol-lowering action.

The study described below demonstrated that pitavastatin attenuated the upregulation of PAI-1 gene in restraint-stressed mice. Twelve to sixteen-month-old male C57BL/6 J mice were administered orally 10 mg/kg/day of pitavastatin or atorvastatin for 3 weeks before the animals were received restraint stress. The dosage of agents we used is regarded to be much excess in comparison with clinical dose because rodents metabolize statins more rapidly than humans. Restraint stress, plasma collection, RNA extraction and quantitative RT-PCR assay were performed, as described previously [3]. PAI-1 antigen levels in plasma were quantified by a sandwich ELISA, as described previously [6]. All procedures were carried out according to the protocol approved by the Animal Care and Use Committee of Nagoya University. Twenty hours of restraint stress to mice caused a substantial induction of PAI-1 antigen in plasma and of PAI-1 mRNA in the liver and adipose tissues, which have been regarded as major sources of PAI-1 [3]. PAI-1 antigen in plasma was dramatically elevated after a 20 h-restraint stress, but this increase attenuated by 40% in mice pretreated with pitavastatin (Fig. 1, left panel). Free PAI-1 activity measured by t-PA binding assay was also elevated by stress and its increase was attenuated by pretreatment with pitavastatin in parallel with PAI-1 antigen level (not shown). Although t-PA antigen levels measured by ELISA were elevated after restraint stress, the degree of elevation (by 2-fold, not shown) was much smaller than PAI-1 induction (by 7-fold), showing that a prothrombotic state was induced by restraint stress. Pitavastatin also suppressed the induction of PAI-1 mRNA by restraint stress in the liver and adipose tissues about 60% of the control (i.e., pitavastatin naive) mice (Fig. 1, middle and right panels), while atorvastatin did not (not shown). As plasma cholesterol levels were not affected by statins in these mice (not shown), pitavastatin may suppress the upregulation of PAI-1 gene independent of its cholesterol-lowering action in restraint-stressed mice. It has been reported that statins reduce the PAI-1 expression by

K. Yamamoto · K. Takeshita · T. Kojima · J. Takamatsu
Department of Transfusion Medicine,
Cardiology and Hematology,
Nagoya University Hospital, Nagoya, Japan

K. Yamamoto (✉)
Department of Transfusion Medicine,
Nagoya University Hospital, 65 Tsurumai,
Showa, Nagoya 466-8560, Japan
e-mail: kojiy@med.nagoya-u.ac.jp

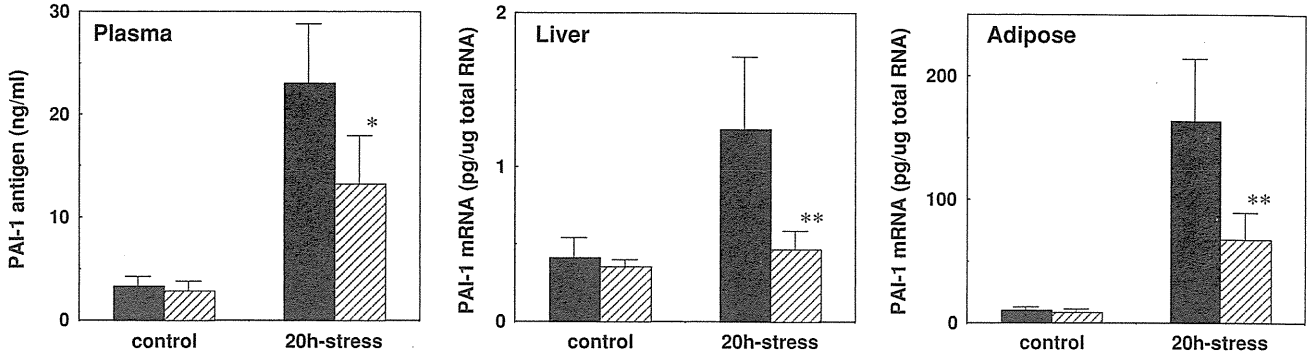

Fig. 1 Twelve to sixteen-month-old mice were administered pitavastatin (10 mg/kg/day) for 3 weeks ($n = 6$, respectively), followed by 20-h-restraint stress. As a control group, non-stressed mice and 20-h-stressed mice without pitavastatin administration were prepared ($n = 6$, respectively). The plasma was collected and measured for PAI-1 antigen (ng/ml) by ELISA assay. Liver and adipose tissue were harvested and analyzed for PAI-1 mRNA (pg/μg total tissue RNA) by competitive RT-PCR. *Closed bars* control (pitavastatin naive) group, *hatched bars* pitavastatin-treated group. The data are presented as the mean and SD. $^*P < 0.05$, $^{**}P < 0.02$ (statistically analyzed by one-way ANOVA)

suppressing the formation of geranylgeranylated proteins required for the proper synthesis of PAI-1 [7], and this may be one of the mechanisms by which pitavastatin attenuates the PAI-1 induction in restraint-stressed mice.

Several differences are observed in the pleiotropic effects of statins. Pitavastatin may more strongly suppress the molecular responses against stress insults, which include the induction of cytokine-induced nuclear factor-κB (NF-κB) and the production of oxidative stress markers in the ischemic model, in comparison with atorvastatin [8, 9]. The expression of PAI-1 gene is upregulated by oxidative stress markers (e.g., 4-hydroxynonenal and 8-hydroxy-2′-deoxyguanosine) [10] and NF-κB, both of which could be induced by stress-related inflammatory cytokines (e.g., TNF-α). Taken together, it is speculated that pitavastatin may attenuate the stress-induced PAI-1 expression through the inhibition of TNF-α-induced NF-κB activation and its anti-oxidative effect. Although there have been some reports on the inhibitory effect of atorvastatin on PAI-1 expression in vitro or ex vivo [11], this agent may have less anti-oxidant potential and less ability to block NF-κB activation than pitavastatin [12], resulting in the lack of suppressive effect on the stress-induced PAI-1 expression. Finally, the finding in this study suggests that pitavastatin contributes, in part, to the prevention of thrombotic cardiovascular diseases associated with physicopsychological stress although further studies are required to elucidate its mechanism.

References

1. Jern C, Eriksson E, Tengborn L, Risberg B, Wadenvik H, Jern S. Changes of plasma coagulation and fibrinolysis in response to mental stress. Thromb Haemost. 1989;62:767–71.
2. Glavin GB, Pare WP, Sandbak T, Bakke HK, Murison R. Restraint stress in biomedical research: an update. Neurosci Biobehav Rev. 1994;18:223–49. doi:10.1016/0149-7634(94)90027-2.
3. Yamamoto K, Takeshita K, Shimokawa T, Yi H, Isobe K, Loskutoff DJ, et al. Plasminogen activator inhibitor-1 is a major stress-regulated gene: implications for stress-induced thrombosis in aged individuals. Proc Natl Acad Sci USA. 2002;99:890–5. doi:10.1073/pnas.022608799.
4. Samad F, Loskutoff DJ. Tissue distribution and regulation of plasminogen activator inhibitor-1 in obese mice. Mol Med. 1996;2:568–82.
5. Markle RA, Han J, Summers BD, Yokoyama T, Hajjar KA, Hajjar DP, et al. Pitavastatin alters the expression of thrombotic and fibrinolytic proteins in human vascular cells. J Cell Biochem. 2003;90:23–32. doi:10.1002/jcb.10602.
6. Yamada T, Takagi A, Takeshita K, Yamamoto K, Ito M, Matsushita T, et al. Enzyme immunoassay for measurement of murine plasminogen activator inhibitor-1, employing a specific antibody produced by DNA vaccines method. Thromb Res. 2003;111:285–91. doi:10.1016/j.thromres.2003.09.007.
7. Essig M, Nguyen G, Prié D, Escoubet B, Sraer JD, Friedlander G. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors increase fibrinolytic activity in rat aortic endothelial cells. Role of geranylgeranylation and Rho proteins. Circ Res. 1998;83:683–90.
8. Hayashi T, Hamakawa K, Nagotani S, Jin G, Li F, Deguchi K, et al. HMG CoA reductase inhibitors reduce ischemic brain injury of Wistar rats through decreasing oxidative stress on neurons. Brain Res. 2005;1037:52–8. doi:10.1016/j.brainres.2004.12.051.
9. Tounai H, Hayakawa N, Kato H, Araki T. Immunohistochemical study on distribution of NF-kappaB and p53 in gerbil hippocampus after transient cerebral ischemia: effect of pitavastatin. Metab Brain Dis. 2007;22:89–104. doi:10.1007/s11011-006-9040-3.
10. Oszajca K, Bieniasz M, Brown G, Swiatkowska M, Bartkowiak J, Szemraj J. Effect of oxidative stress on the expression of t-PA, u-PA, u-PAR, and PAI-1 in endothelial cells. Biochem Cell Biol. 2008;86:477–86. doi:10.1139/O08-137.
11. Li JQ, Zhao SP, Li QZ, Cai YC, Wu LR, Fang Y, et al. Atorvastatin reduces plasminogen activator inhibitor-1 expression in adipose tissue of atherosclerotic rabbits. Clin Chim Acta. 2006;370:57–62. doi:10.1016/j.cca.2006.01.024.
12. Hilgendorff A, Muth H, Parviz B, Staubitz A, Haberbosch W, Tillmanns H, et al. Statins differ in their ability to block NF-kappaB activation in human blood monocytes. Int J Clin Pharmacol Ther. 2003;41:397–401.

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LETTER TO THE EDITOR

Severe hemophilia A in a Japanese female caused by an *F8*-intron 22 inversion associated with skewed X chromosome inactivation

Yuhri Miyawaki · Atsuo Suzuki · Yuhta Fujimori · Akira Takagi · Takashi Murate · Nobuaki Suzuki · Akira Katsumi · Tomoki Naoe · Koji Yamamoto · Tadashi Matsushita · Junki Takamatsu · Tetsuhito Kojima

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Hemophilia A is an X-linked recessive bleeding disorder with a worldwide prevalence of approximately 1 in 5,000 males. Hemophilia A is caused by a deficiency or functional defect in coagulation factor VIII (FVIII), and its clinical severity is inversely related to residual FVIII activity (FVIII:C). Patients with less than 1, 1–5, and 5–30% FVIII:C are classified as having severe, moderate, and mild hemophilia A, respectively [1]. The gene encoding FVIII (*F8*) is located in the most distal region of the long arm of the X chromosome (Xq28) and spans 186 kb [2]. The molecular basis underlying hemophilia A is well characterized, and various causative defects, such as point mutations, insertions, deletions and other genetic abnormalities, have been found in the *F8* gene of hemophilia A patients. Among them, a large genomic inversion disrupting *F8* at intron 22 (*F8-int22* inversion) is found in about half of severe hemophilia A cases including Japanese [3–5], and an inversion at intron 1 (*F8-int1* inversion) is found in 1–5% of cases [6, 7].

Hemophilia A affects males, and is transmitted by heterozygous females who are denoted as carriers. They are usually asymptomatic, because their proportion of somatic cells with an inactivated normal X chromosome is approximately equal to the proportion with an inactivated mutated X chromosome [8]. However, there are several potential genetic mechanisms leading to the phenotypic expression of very low FVIII:C in female carriers as hemophiliacs. Thus, in rare cases, severe hemophilia A can occur in females homozygous (e.g., consanguinity) or compound heterozygous for mutations in *F8* [9, 10], through X chromosome abnormalities such as monosomy X (45 X, Turner syndrome), and due to skewed X inactivation in a heterozygous female carrier [11, 12]. In this study, we investigated the genetic mechanisms of *F8* defects to elucidate the molecular pathogenesis responsible for severe hemophilia A in a Japanese female. The study was approved by the Ethics Committee of the Nagoya University School of Medicine, and genomic DNA samples from all participants were isolated from peripheral leukocytes by phenol extraction as described previously [13], after informed consents were obtained.

The patient was a 21-year-old female and suffered from bleeding symptoms, such as easy bruising and joint swelling, since she was 2 years old. She was diagnosed as a severe hemophilia A (FVIII:C $< 1\%$ and FVIII:Ag $< 5\%$), and received FVIII concentrates as replacement therapy. However, she had developed hemophiliac arthropathy in her left elbow joint. Her elder brother suffered from similar bleeding symptoms and had also been diagnosed as a severe hemophilia A (FVIII:C $< 1\%$ and FVIII:Ag $< 5\%$).

Y. Miyawaki · A. Suzuki · Y. Fujimori · A. Takagi · T. Murate · T. Kojima
Department of Pathophysiological Laboratory Sciences, Nagoya University Graduate School of Medicine, Nagoya, Japan

A. Takagi · T. Murate · T. Kojima (✉)
Department of Medical Technology, Nagoya University School of Health Sciences, 1-1-20 Daiko-Minami, Higashi-ku, Nagoya 461-8673, Japan
e-mail: kojima@met.nagoya-u.ac.jp

N. Suzuki · A. Katsumi · T. Naoe
Department of Hematology-Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan

K. Yamamoto · T. Matsushita
Division of Transfusion Medicine, Nagoya University Hospital, Nagoya, Japan

J. Takamatsu
Aichi Red Cross Blood Center, Seto, Japan

The mother was a hemophiliac carrier (FVIII:C = 48% and FVIII:Ag = 61%). The levels of von Willebrand factor antigen and ristocetin cofactor activity in all family members tested including the patients were within the normal range. A DNA sample from the father was not available. Chromosomal analysis of the patient revealed a normal female 46, XX karyotype with no structural abnormalities (data not shown).

The pedigree of the patient's family is shown in Fig. 1. First, we analyzed inversions of *F8-int22* and *F8-int1* by the inverse shifting-polymerase chain reaction (IS-PCR) approach [14], and found that the patient was heterozygous for *F8-int22* inversion type I mutation (data not shown). We detected the same *F8-int22* inversion in her brother monozygously and in her mother heterozygously. These results indicated the *F8-int22* inversion found in the patient to be inherited from her mother. We also tested for the *F8-int22* inversion in their DNA by Southern blotting [15] as well as by single-tube long-distance PCR (LD-PCR) [6, 16], and obtained consistent results with the IS-PCR data (data not shown).

Female carriers with a heterozygous abnormal *F8* gene show generally about 50% of FVIII:C due to random inactivation of the X chromosomes with a rate equivalence. The patient was diagnosed as a hemophilia A carrier with the *F8-int22* inversion in terms of genotype, but with a severe hemophilia A phenotype. Because the skewed X chromosome inactivation in a heterozygous carrier is known to be a cause of X-linked recessive disorders in females [8], we

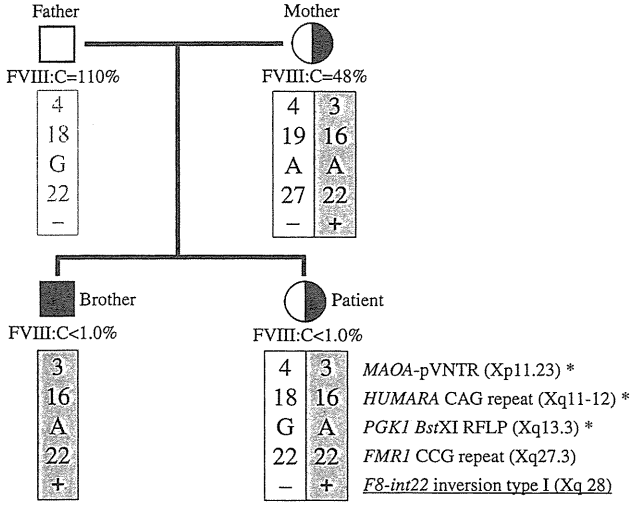

Fig. 1 Pedigree and X chromosome haplotypes of the family members. FVIII:C levels (%) were represented *below the symbols*. Haplotypes of the X chromosomes were shown in *boxes*, and represented the number of repeats, individual nucleotides, or presence/absence (+/−) of the mutation at each locus. A predicted haplotype of the father, for whom data were unavailable, was shown in *bright gray*. *Informative markers for the assessment of the X chromosome inactivation pattern of the patient

tried to examine the DNA methylation pattern at the heterozygous X-linked gene loci. First, we determined the chromosome haplotype of the family members, and found that three markers, the monoamine oxidase A gene (*MAOA*, Xp11.23) promoter VNTR (*MAOA*-pVNTR) [17], the human androgen receptor gene (*HUMARA*, Xq11–12) CAG repeat [18] and the phosphoglycerate kinase 1 gene (*PGK1*, Xq13.3) *Bst*XI polymorphism [19], were informative, but the fragile X mental retardation gene 1 (*FMR1*, Xq27.3) CGG repeat [20] was not (Fig. 1).

Among them, we assessed the DNA methylation pattern at *MAOA*-pVNTR as shown in Fig. 2. Since *Hpa*II cleaves non-methylated DNA in the active X chromosome, PCR amplification of the gene in DNA treated with *Hpa*II will fail. In the sample of the patient's brother having a single active X chromosome, PCR-amplified fragments appeared as a 210-bp band (3-repeats allele, lane 5), but disappeared on digestion of the template DNA with *Hpa*II (lane 6). The analysis of PCR-amplified fragments from the mother's DNA samples with or without digestion by *Hpa*II showed two distinct bands, a 4-repeats allele (240-bp) and a 3-repeats allele (210-bp) (lanes 3 and 4), suggesting that random X inactivation occurred in her somatic cells. In contrast, the DNA of the patient not digested with *Hpa*II gave two distinct bands (lane 1), a 4-repeats allele from the father (240-bp) and a 3-repeats allele from the mother (210-bp), but only the 240-bp band was observed on PCR amplification of the *Hpa*II-digested DNA sample (lane 2). Analysis of this pattern with NIH image version 1.62 revealed an extremely skewed inactivation of the paternally derived X chromosome containing a normal *F8* (ratio 99.5: 0.5), fully consistent with the patient's severe hemophilia A phenotype. We also analyzed X chromosome inactivation patterns at the other two gene markers, the *HUMARA* CAG repeat and the *PGK1 Bst*XI polymorphism, and obtained similar findings with skewed inactivation rates (data not shown). Meanwhile, we analyzed the *F8* genomic sequence of the patient by PCR-mediated direct sequencing, and found no abnormality causing a FVIII deficiency (data not shown). Taken together, it was suggested that an extremely skewed inactivation toward the paternally derived X chromosome carrying a normal *F8* gene could occur in this female hemophilia A patient.

X chromosome inactivation is a process in mammals by which one of the two X chromosomes in female somatic cells is inactivated to eliminate differences between males and females in levels of expression of the genes on the X chromosome [21]. The determination of whether the maternally or paternally derived X chromosome is to be inactivated is random, and the inactive X chromosome is silenced by CpG hyper-methylation. X chromosome inactivation is regulated by the expression of the X-inactive-specific transcript gene (*XIST*) [22]. The *XIST* antisense

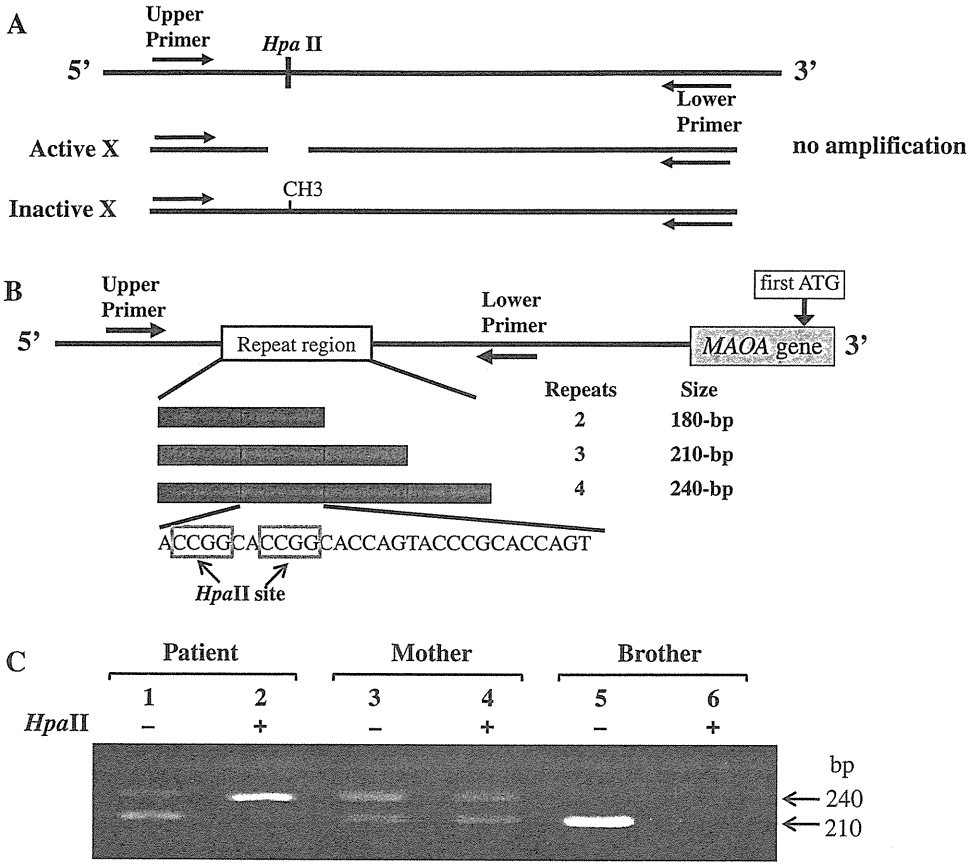

Fig. 2 Assessment of X chromosome inactivation at *MAOA*-pVNTR. **a** Scheme of methylation-sensitive *Hpa*II-PCR amplification at *MAOA*-pVNTR. The symbol (CH3) indicated a methylation site in the *Hpa*II cleavage site of the gene. Since *Hpa*II cleaves non-methylated DNA in the active X chromosome, PCR amplification of the *Hpa*II-treated gene on the active X chromosome will fail. **b** Scheme of the *MAOA* gene and *MAOA*-pVNTR repeat sequence. Repeat structure, sequence of the repeat region, allele (repeat) numbers, and PCR product sizes were shown. *Solid arrows* indicate a PCR primer set. **c** Methylation-sensitive *Hpa*II-PCR patterns at *MAOA*-pVNTR. After digestion of the DNA with *Hpa*II, PCR-mediated *MAOA*-pVNTR for the patient showed an extremely unbalanced amplification toward the paternal 4-repeats allele. Comparatively, that for the mother showed nearly equal levels of amplification for both alleles. +: *Hpa*II-predigested DNA, −: undigested DNA

RNA (*TSIX*), which is an RNA gene and a negative regulator of *XIST*, is also related to this process [23]. A skewed X chromosome can occur as a result of chance, abnormalities in these factors, or selection that eliminates the normal X chromosome after X inactivation [8]. We did not elucidate the precise mechanisms responsible for the skewed X inactivation in this patient; however, it might be possible due to an aberration of these factors.

In conclusion, the maternally transmitted *F8-int22* inversion and the extremely skewed inactivation of the paternally derived X chromosome carrying a normal *F8* would cause the severe hemophilia A phenotype in this female patient, although the presence of other mutation cannot be excluded completely.

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References

1. White GC II, Rosendaal F, Aledort LM, Lusher JM, Rothschild C, Ingerslev J, et al. Definitions in hemophilia. Recommendation of the scientific subcommittee on factor VIII and factor IX of the scientific and standardization committee of the International Society on Thrombosis and Haemostasis. Thromb Haemost. 2001;85:560.
2. Gitschier J, Wood WI, Goralka TM, Wion KL, Chen EY, Eaton DH, et al. Characterization of the human factor VIII gene. Nature. 1984;312:326–30.
3. Lakich D, Kazazian HH, Antonarakis SE, Gitschier J. Inversions disrupting the factor VIII gene are a common cause of severe haemophilia A. Nat Genet. 1993;5:236–41.
4. Naylor J, Brinke A, Hassock S, Green PM, Giannelli F. Characteristic mRNA abnormality found in half the patients with

severe haemophilia A is due to large DNA inversions. Hum Mol Genet. 1993;2:1773–8.
5. Fukuda K, Naka H, Morichika S, Shibata M, Tanaka I, Shima M, et al. Inversions of the factor VIII gene in Japanese patients with severe hemophilia A. Int J Hematol. 2004;79:303–6.
6. Bagnall RD, Waseem N, Green PM, Giannelli F. Recurrent inversion breaking intron 1 of the factor VIII gene is a frequent cause of severe hemophilia A. Blood. 2002;99:168–74.
7. Cumming AM. The factor VIII gene intron 1 inversion mutation: prevalence in severe hemophilia A patients in the UK. J Thromb Haemost. 2004;2:205–6.
8. Puck JM, Willard HF. X inactivation in females with X-linked disease. N Engl J Med. 1998;338:325–8.
9. Cai X, Wang X, Dai J, Fang Y, Ding Q, Xie F, et al. Female hemophilia A heterozygous for a de novo frameshift and a novel missense mutation of factor VIII. J Thromb Haemost. 2006; 4:1969–74.
10. Windsor S, Lyng A, Taylor S, Ewenstein B, Neufeld E, Lillicrap D. Severe haemophilia A in a female resulting from two de novo factor VIII mutations. Br J Haematol. 1995;90:906–9.
11. Favier R, Lavergne J-M, Costa J-M, Caron C, Mazurier C, Viemont M, et al. Unbalanced X-chromosome inactivation with a novel FVIII gene mutation resulting in severe hemophilia A in a female. Blood. 2000;96:4373–5.
12. Valleix S, Vinciguerra C, Lavergne J-M, Leuer M, Delpech M, Negrier C. Skewed X-chromosome inactivation in monochorionic diamniotic twin sisters results in severe and mild hemophilia A. Blood. 2002;100:3034–6.
13. Kojima T, Tanimoto M, Kamiya T, Obata Y, Takahashi T, Ohno R, et al. Possible absence of common polymorphisms in coagulation factor IX gene in Japanese subjects. Blood. 1987;69:349–52.
14. Rossetti L, Radic C, Larripa I, De Brasi C. Developing a new generation of tests for genotyping hemophilia-causative rearrangements involving *int22h* and *int1h* hotspots in the factor VIII gene. J Thromb Haemost. 2008;6:830–6.
15. Sugihara T, Takahashi I, Kojima T, Okamoto Y, Yamamoto K, Kamiya T, et al. Identification of plasma antibody epitopes and gene abnormalities in Japanese hemophilia A patients with factor VIII inhibitor. Nagoya J Med Sci. 2000;63:25–39.
16. Liu Q, Nozari G, Sommer SS. Single-tube polymerase chain reaction for rapid diagnosis of the inversion hotspot of mutation in hemophilia A. Blood. 1998;92:1458–9.
17. Sabol SZ, Hu S, Hamer D. A functional polymorphism in the monoamine oxidase A gene promoter. Hum Genet. 1998; 103:273–9.
18. Allen RC, Zoghbi HY, Moseley AB, Rosenblatt HM, Belmont JW. Methylation of *Hpa*II and *Hha*I sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. Am J Hum Genet. 1992;51:1229–39.
19. Okumura K, Fujimor Y, Takagi A, Murate T, Ozeki M, Yamamoto K, et al. Skewed X chromosome inactivation in fraternal female twins results in moderately severe and mild haemophilia B. Haemophilia. 2008;14:1088–93.
20. Lee S-T, McGlennen RC, Litz CE. Clonal determination by the fragile X (FMR1) and phosphoglycerate kinase (PGK) genes in hematological malignancies. Cancer Res. 1994;54:5212–6.
21. Lyon M. Gene action in the X-chromosome of the mouse (*Mus musculus* L.). Nature. 1961;190:372–3.
22. Hoki Y, Kimura N, Kanbayashi M, Amakawa Y, Ohhata T, Sasaki H, et al. A proximal conserved repeat in the Xist gene is essential as a genomic element for X-inactivation in mouse. Development. 2009;136:139–46.
23. Lee J, Davidow LS, Warshawsky D. Tsix, a gene antisense to Xist at the X-inactivation centre. Nat Genet. 1999;21:400–4.

大量出血時の病態と輸血療法
——フィブリノゲン濃縮製剤投与の有用性

Pathomechanisms of coagulopathy in massive bleeding and fibrinogen concentrate substitution therapy

高松純樹
Junki TAKAMATSU
愛知県赤十字血液センター

◎生体で認められる出血には，主として血管の破綻による物理的出血(局所出血)と，止血に関する血液成分の異常のために起こる全身性出血傾向による．したがってそれに対する対応としては，物理的出血では局所処置(局所止血)が第一義的であり，いくら血小板や血漿製剤に投与を行っても止血はできない．一方，その出血の原因が止血に関する血小板，凝固因子およびその制御因子などの欠乏または機能異常による場合には，適切な検査を参考にして欠乏あるいは機能異常を呈している成分(ときには複数)の十分な補充が必要である．とくに術中の大量出血時では凝固因子，血小板の漏出，消費，枯渇が惹起され，さらに大量出血という悪循環に陥る．なかでもフィブリノゲンは凝固系の最終的な基質でありながら，もっとも早期に止血レベル以下に減少することから，もっとも早期でかつ十分な補充療法が求められる．

Key word：大量出血，凝固障害，フィブリノゲン製剤，フィブリノゲン欠乏症，輸血療法

24 時間以内に循環血液量あるいはそれを超える出血を大量出血と定義しているが，これらの原因となるのは外傷，消化管出血，心臓血管外科手術(とくに胸部大血管手術)，悪性腫瘍(とくに肝胆道系腫瘍)，さらには産科的疾患が含まれている．医療施設によってはこれらに加えて肝移植術がその原因としてあげられ，名古屋大学では 2005～2006 年には平均 50 単位の赤血球輸血が行われていた．本稿では，このような大量出血時の輸血療法について述べる．

大量出血の病態生理

大量出血時においては，血小板数の減少，機能異常や凝固異常がしばしば認められ，その発症については以下に述べるいくつかの要因が考えられている．

その第 1 は，大量出血時には多くの凝固因子が喪失・消費され，その結果として止血に必要な凝固因子や，ときには血小板までが枯渇する状態となる．それに対して輸血される全血製剤あるいは

サイドメモ 1　止血系におけるフィブリノゲンの役割とその病態

フィブリノゲンは肝で産生される分子量約 34 万の血漿糖蛋白質で，止血系における最終基質である．さらに，フィブリノゲンは血小板凝集に必須の蛋白であり，さらに妊娠，とくに着床および胎児生育にとって必須である．病的状態では先天性無・低フィブリノゲン血症患者は終生の出血傾向を有する．一方，後天性低フィブリノゲン血症は本稿でのべる大量出血時以外にも肝障害患者のような産生障害あるいは線溶亢進によって起こる．線溶亢進状態には，全身性の凝固反応なしで起こる一次線溶と，血管内凝固症候群(DIC)のような二次線溶がある．前者では血栓溶解療法，あるいはある種の悪性腫瘍や肝障害でみられる．いかなる原因であれ，DIC では二次線溶を惹起するが，頭部外傷，前置胎盤，大動脈瘤破裂あるいは急性前骨髄性白血病などではさらに線溶亢進状態がみられる．このようにフィブリノゲンは種々の生理的役割を担うとともに，欠乏状態では不断の出血の原因となる．

赤血球濃厚液では血小板や凝固因子が十分含有されていないために，赤血球の補充のみで結果的に血小板，凝固因子あるいはその両方が欠乏し，希釈性の凝固障害が引き起こされることとなる．血小板についていえば，24時間以上冷蔵保存された全血製剤，濃厚赤血球製剤中には機能を有した血小板は存在していない．全血製剤では多くの凝固因子は比較的よく保たれているが，第Ⅷ，第Ⅴ因子は4℃で21日保存されるとすると，活性はそれぞれ5%，35%と，著しい低下がみられる．赤血球濃厚液においては血漿成分の含有はほとんどない．

ブタを用いた動物実験モデルにおいても，出血自体は中等度であっても，増加しているフィブリノゲン産生は分解あるいは消費されるフィブリノゲンを補うことはできず，ついには著しい出血という悪循環となることが明らかにされている[1]．

第2は，血管内凝固症候群(DIC)による凝固因子の消費による凝固異常が考えられる．広範な組織障害が起こり，循環血液量の減少や酸素運搬能が適切に補正されなければ組織は虚血あるいは壊死状態となり，その結果，大量の組織因子の放出によりDICが惹起されることとなる．凝固異常の程度と血圧の低下時間の間には密接な関係があるという報告からも，この病態が大量輸血時の凝固異常の原因として重要な役割を担っていることは明らかである．

第3は，大量に投与される晶質液，あるいは膠質液による希釈の影響や，低体温による血小板機能低下あるいは凝固反応の低下も考えられる．とくに膠質液はフィブリノゲンがフィブリンへ転換する過程であるfibrin monomerの重合反応に阻害的に作用し，結果としてフィブリノゲン値を低下させることにもなる[2]．

1．血小板減少

大量輸血時(通常は循環血液量を超える輸血量)に対して全血製剤の投与のみでは血小板数は5万～10万程度に低下することもまれではない．しかし，輸血量と血小板減少が一定ではないことは，大量輸血患者に対して一定の割合での血小板予防投与はかならずしも有効とは限らないことを示唆する．すなわち，血小板減少即細血管性出血を意味するわけではなく，また減少程度が出血の程度の予測になるとも限らないからである．

2．凝固因子欠乏

前述したように全血輸血では第Ⅷ，第Ⅴ因子以外は比較的保たれているために大量輸血時には全血輸血が適応となることもあるが，赤血球濃厚液のみの補充では凝固異常は必発である．しかし，凝固に関する検査異常が即出血を示すわけでないことは血小板数と出血の関係と同様である．プロトロンビン時間，あるいは活性化部分トロンボプラスチン時間がコントロールに比べて1.5倍以上延長，あるいはフィブリノゲン値が75 mg/dl以下では出血の可能性がある．なかでもフィブリノゲンは，減少する凝固因子になかでももっと早く止血レベル以下になることが報告されている[3]．

図1に大量出血時の凝固異常のメカニズムを示す．もともと出血傾向のある場合であっても出血傾向のない場合であっても出血量が2～3 lに達すると，軽度ではあるが凝固異常が起こりはじめる．その結果，血小板・凝固因子の漏出が生じる．生体での血小板，凝固因子の数・量の変動(この場合は減少となる)があってもただちに凝固異常に陥

サイドメモ2　大量出血に対する活性化リコンビナント第Ⅶ因子(rⅦa)の意義

凝固因子に対するインヒビターを生じた血友病患者にみられる急性期の出血に対する治療製剤として開発された活性化リコンビナント第Ⅶ因子(rⅦa)が，重症外傷患者や産科出血を含む大量出血に対する治療に有用であるという報告がある．rⅦaは生理的濃度で，活性化された血小板に結合し，トロンビン産生を爆発的に起こすことにより安定化フィブリンを形成する．rⅦaが有効に作用するためには，フィブリノゲン値がすくなくとも100 mg/dl以上，血小板数は50,000以上が必要である．言い換えれば，rⅦaはトロンビン生成を亢進させるのであって，すべての出血を止めるわけではなく，フィブリノゲン，血小板なくしてはその機能を発揮することはできない．さらに，現時点ではどのような出血患者にどのような量をどのような間隔で投与すべきかという成績はきわめて少なく，今後の課題である．とくに大量投与では血栓症のおそれがあるところから，安易な使用は厳に慎むべきである．

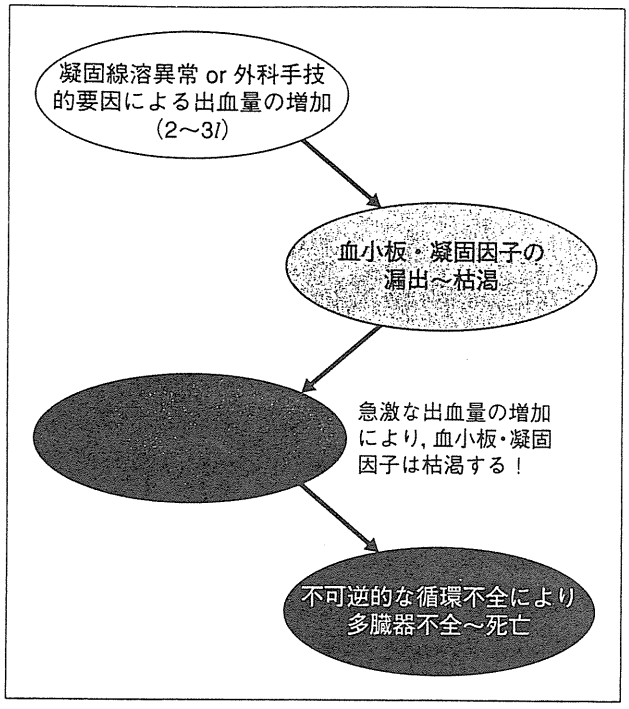

図 1　大量出血による凝固異常

るわけではなく，血小板ではプールされていた分が動員されたり，凝固因子では肝での産生が亢進することになる．しかし，このような代償もできなくなるとこれらは枯渇し，一気に大量出血となる．このような状態はさらに出血を増強させ，ついには多臓器不全，果てには死亡となる．また，外傷性の大量出血にあってはショック，挫滅組織からの組織因子の流入による凝固活性化，さらには抗凝固系や線溶系経路の活性化などが複合的に加わること，治療・病態の推移などによるアシドーシス，低体温，血液の希釈，あるいは心機能低下による拍出障害，凝固因子の消費など，手術時にはみられない要因も考えられる[4]．

いずれにせよ，大量出血により急速な凝固異常を生じることはその基礎疾患・病態によらないことに留意し，適切かつ迅速な対応が求められる．

大量出血に対する輸血療法

出血に対する治療の大原則は局所止血であることは自明の理であり，また出血の結果として生じるショックに対する治療，組織における虚血，低体温に対する対応などが求められる．それらは救命救急処置としては重要であるが，ここでは主として輸血による治療について詳しく述べることとする．

大量輸血時における凝固異常に際してはまず止血検査を行い，以後止血が完了するまで適宜行うことが必要である．プロトロンビン時間あるいはINR，活性化部分トロンボプラスチン時間(APTT)，フィブリノゲン値，血小板数は必須の検査項目であり，なかでもフィブリノゲン値と血小板数の変動はきわめて重要であることに留意すべきである．

しかし，止血検査に異常があるからといって予防的な血小板・血漿輸血の適応はないことから，輸血をする判断には実際の出血状況，バイタルサインなど総合的にモニタリングをすることが重要である．

輸血療法の実際

1．赤血球

赤血球それ自体は止血に直接的に関与してはいないが，血小板の遊走に対する流体力学的効果により活性化血小板の機能を亢進し，またトロンビン産生にも寄与している．急速にヘマトクリット値が低下すると出血時間が延長することは知られているが，これは赤血球膜表面に存在するエラスターゼが第Ⅸ因子を活性化し，止血に関与していることと関係しているものと推定されている．しかし，現時点でも大量出血患者でこのような効果をもたらすためには，どの程度のヘマットクリット値やヘモグロビン値が適切であるかははっきりしていない．

一方，外傷患者での大量出血例への赤血球輸血は死亡率を高めているとか，肺障害をもたらすとか，感染症に陥りやすいとか，あるいは腎不全を惹起しやすいという報告もあり，とくに採血後2週間以上たった製剤では高率との報告がある[5-7]．

2．フィブリノゲン

フィブリノゲンは大量出血時においてもっとも早期に止血レベルを下まわる因子であり，大量出血時の鍵となる止血に関する要因といってよいことはすでに述べた．また，産科的出血時においてはフィブリノゲン値は大出血を予知する唯一因子であり，患者のフィブリノゲン値が200 mg/dl以下であれば(妊婦であるから本来ならば400〜500

mg/dl 程度であり，この値は明らかに低い)100%の予知率である[8]．

このように，大量出血に際してフィブリノゲンを十分補充する必要性・有効性は，臨床例のみならず，ブタを用いた検討によっても確認されている[9]．フィブリノゲンを補うには，①新鮮凍結血漿(FFP)，②クリオプレチピテート(クリオ)，③フィブリノゲン濃縮製剤が，選択肢としてある．

① **FFP**……FFP はすべての凝固因子を含んでおり，わが国においては各種凝固因子の補充に加えてフィブリノゲンの補充に唯一保険適応となっている．しかし，フィブリノゲン濃度を FFP にて止血の最低値から止血レベルに上昇させることは，FFP に含まれるフィブリノゲン値は正常血漿と同値あるいは抗凝固剤の影響で正常血漿以下であることから 10～20 ml/kg と大量投与が必要となる．さらに，輸血関連肺障害(TRALI)，アナフィラキシー反応，場合によっては溶血反応も起こりうる．

② **クリオ**……もうひとつの選択肢としてのクリオは，以下のようにして作製される．新鮮凍結血漿(FFP)を 4℃の冷蔵庫内にて 1～2 日間かけてゆっくりと解凍して遠心操作すると，クリオプレシピテートと称される沈殿物とクリオスーパーネータントと称される上清が得られる．この沈殿物には，第Ⅷ因子や第XIII因子など分子量の大きな凝固蛋白やフィブリノゲン，フォンビレブランド因子，フィブロネクチンなどの接着性蛋白が含まれている．上製分画を除去後，沈殿物であるクリオ分画を再度 37℃に加温すると速やかに溶解するので，これを再凍結しておけば 1 年間は有効である．5 単位の FFP からは容量として約 10 倍以上濃縮され，1 g 前後のフィブリノゲンが得られる．かつては血友病治療に用いられていたが，現在ではもっぱらフィブリノゲンの補充療法用にアメリカを中心に用いられているが，ヨーロッパでは安全な濃縮フィブリノゲン製剤が入手されるようになり使用は激減している．作製には数日かかるので，緊急用にはあらかじめ ABO 型別あるいは万人用に AB 型のクリオを作成しておかねばならないこと，ドナーのフィブリノゲン濃度に依存するので，濃度が一定しないこととウイルスの不活化がされていない点は濃縮製剤に比べて大きなマイナス点である．

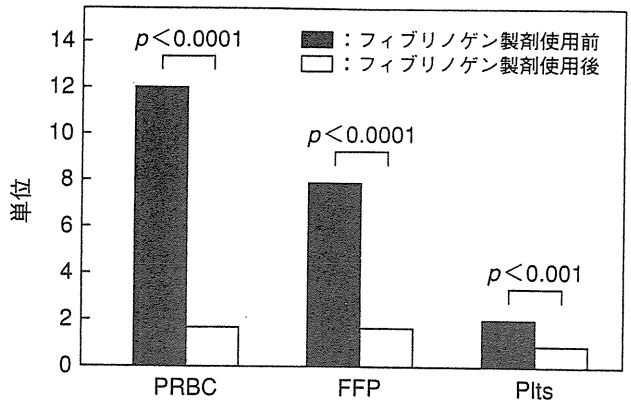

図 2　血液製剤の使用量の変化[10]

③ **フィブリノゲン濃縮製剤**……フィブリノゲン濃縮製剤はわが国では先天性無あるいは低フィブリノゲン血漿患者の補充療法のみが保険適応で，後天性低フィブリノゲン血症には保険適応となっていない．欧米，とくにヨーロッパでは先天性のみならず，後天性低フィブリノゲン血症にも使用されており，多くの報告例がある[10]．図 2 に，大量出血後の低フィブリノゲンに対するフィブリノゲン製剤の補充成績の一部を示す．患者は，参加出血患者，小児科，胸部大動脈血管瘤，腹部大動脈瘤，外傷患者など 43 名の低フィブリノゲンに対して濃縮フィブリノゲン製剤を投与し，その前後の製剤の輸血量を比較した．成人では平均 4,000 ml の輸血が 50 ml へと著減したが，小児例でも輸血必要量は減少したが，統計学的には有意差はなかった．同様に，種々の低フィブリノゲン血症に対する濃縮フィブリノゲン製剤の有効性についての報告がなされている[11,12]

名古屋大学病院においても，大出血をきたす疾患の手術に際しては濃縮フィブリノゲン製剤を使用しているが，2 つのその代表的疾患の経時的な平均出血量と平均輸血量を示す(図 3)．2005～2006 年はほとんど濃縮フィブリノゲン，クリオを使用していない時期であり，2008 年は積極的に使用している時期で，2007 年はその中間にあたる．図 3 でも明らかなように，胸部外科手術(ほとんどが胸部大動脈瘤)，肝移植手術において出血量，輸血量ともに半減している．

さて，実際のフィブリノゲンの投与については，

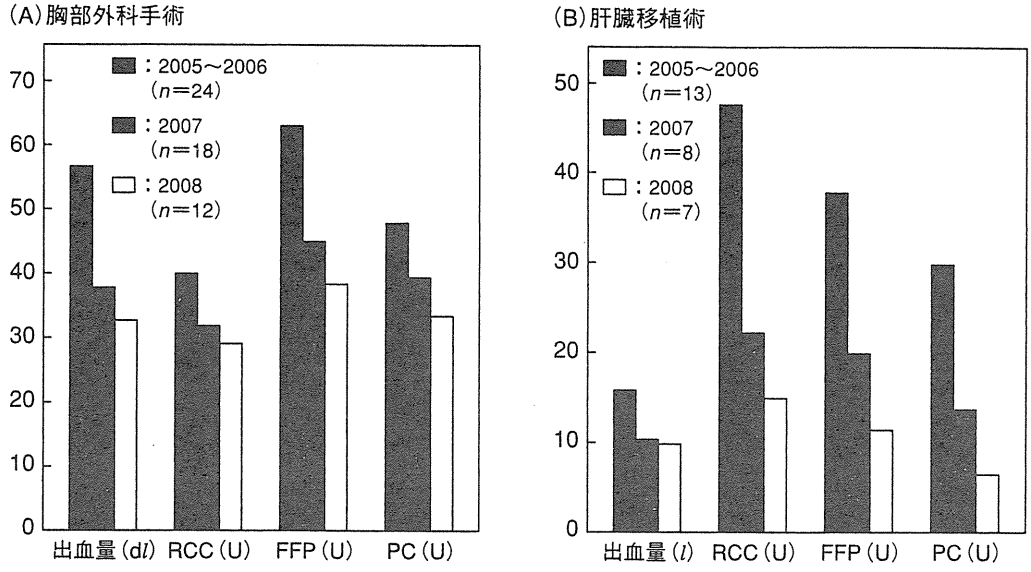

図 3　大量出血(74,000 ml)または大量輸血(RCC720U)症例における出血量・輸血量
(2006～2008)

以下のデータを参考に投与量と時期を考慮する．体重 50 kg の患者では 3 g の投与で理論上 100～120 mg/dl 上昇するが，大出血時では当然のことながら回収率は低下するので，増量する必要がある．フィブリノゲン値が 50 mg/dl 以下では止血不能であり，止血栓形成能ゼロであるのでただちに濃縮フィブリノゲン製剤の投与が必要となる．50～100 mg/dl では出血傾向著明であり，基礎疾患，出血の状況によるが，早期の補充が望まれる．一方，150 mg/dl 前後では止血不良であってただちに大出血となることはないが，出血が持続していれば，容易に 100 mg/dl，50 mg/dl となり大出血に陥るので，フィブリノゲンの補充が望ましい．

3. 血小板

従来は，大量出血時における全身性の細血管性出血がみられたならば，止血検査を行うとともに血小板輸血を考慮することが第一義的と考えられてきた．しかし，すでに述べているように，大量出血時の血小板，凝固因子の変動ではフィブリノゲンがもっとも早期に止血レベル以下に低下すること，生理的な血小板凝集にフィブリノゲンは必須であること，さらにはブタを用いた実験では出血の速度を減少させたり生存期間の延長には，血小板製剤よりもフィブリノゲン製剤のほうが有効であるという報告や[10]，そして 904 例の血小板減少患者の血栓形成能に対するフィブリノゲンの有効性から[11]，血小板は第一義的に考慮すべきではないと思われる．とくに血小板数が 10 万前後であるにもかかわらず出血が持続した場合には，血小板減少による出血ではなく，フィブリノゲンを含む凝固因子の減少，血小板機能異常あるいは DIC のような凝固異常も考慮する．一方，頭部外傷，動脈瘤破裂，ショック状態が持続し長時間虚血状態が持続した場合などでは，とくに DIC による血小板・凝固因子の消費に基づいた出血が顕著にみられることから，どのような製剤が必要か十分に考慮すべきである．

4. FFP

FFP もわが国の産科出血時または大量出血時の際のガイドライン，あるいは PT，APTT がコントロールの 1.5 倍以上に延長した凝固異常で用いられる．その投与量は 10～15 ml/kg の範囲での使用することが推奨されている．しかし，FFP は前述したように，凝固因子の濃度は正常ヒト血漿と同じか低いために，とくにフィブリノゲンの補充という観点では循環負荷に陥る量が必要であること，一気に止血濃度までに上げることができないこと，さらには大量使用ではときに血液型の不適合の心配があること，感染症の伝播のおそれがあること，さらには急性輸血関連肺障害(TRALI)の合併が否定できないことなど，大量出血時のフィブリノゲン補充にはかならずしも適切とはいえな

い.

大量出血時の輸血療法にあたっての考慮すべき点

1. フィブリノゲンの定量

大量出血時には止血に関する因子のうち，フィブリノゲン値がもっとも早期に止血レベルを割り込み，大量出血の悪循環に陥ることはすでに述べ，フィブリノゲン値の測定は重要であると思われる．であるがゆえに，フィブリノゲン値の測定は大出血に陥ってから慌てて行うのではなく，可能性があれば早め早めに行うことが肝心である．

現在，フィブリノゲンの定量はほとんどの医療機関では凝固機器を用いて PT, APTT と同時に測定していると思われる．その原理は，一定のトロンビン濃度(かなりの高濃度)を用いた血漿トロンビン時間はフィブリノゲン値の関数であることに基づいている．すなわち，フィブリノゲン値が高値であれば，トロンビン時間は短く，濃度が低ければ延長するというものである．しかし，大量出血時に投与される代用血漿(とくに膠質液)が以上の測定系に影響し，みかけ上高値となると報告されており[13,14]，これは出血があるにもかかわらず，検査上フィブリノゲン値は高い値が得られるとされ，代用血漿が大量に使用される大量出血時には注意すべき点である．

2. 凝固検査

前項においても述べたように，大量出血時には凝固能のモニターが重要であるが，検査それ自体の問題についても十分配慮する必要がある．大量出血時には血圧の低下などにより末梢血から直接採血することは困難であっても，カテーテルからの採血は検査に影響することが多く，極力さけるべきである．

さらに，迅速な対応ができるようになったとはいえ，凝固検査には一定の時間がかかることは避けられず，得られた結果も 30〜40 分前のものである．したがって，早め早めの検査をすること，フィブリノゲン製剤を含む輸血の判断も早め早めに対応することが重要である．

PT, APTT は FFP の投与にあたって目安になっているが，本来この検査は単独の因子欠損ではそれなりの意義を見出すことができるが，大量出血時のような複雑な凝固異常ではかならずしも適切な指標とはなりえないことに十分留意すべきであり，医療者は臨床的に出血の状況，バイタルサインなども十分考慮して判断すべきである．

文献

1) Martini, W. Z. et al.: *Am. J. Physiol. Endcrinol. Metab.*, **289**: E930-E934, 2005.
2) Fenger-Eriksen, C. et al.: *J. Thromb. Haemost.*, **7**: 1099-1105, 2009.
3) Hippala, S. T. et al.: *Anesth. Analg.*, **81**: 360-365, 1995.
4) Maegele, M. et al.: *Injury*, **38**: 298-304, 2007.
5) Malone, D. L. et al.: *J. Trauma*, **54**: 898-905, 2003.
6) Weinberg, J. A. et al.: *J. Trauma*, **65**: 794-798, 2008.
7) Claridge, J. A. et al.: *Am. Surg.*, **68**: 566-572, 2002.
8) Charbit, B. et al.: *J. Thromb. Haemost.*, **5**: 266-273, 2007.
9) Fries, D. et al.: *Br. J. Anaesth.*, **95**: 172-177, 2005.
10) Fenger-Eriksen, C. et al.: *Br. J. Anaesth.*, **101**: 769-773, 2008.
11) Weinkove, R. et al.: *Transfus. Med.*, **18**: 151-157, 2008.
12) Dane, A. F. et al.: *Vox Sang.*, **94**: 221-226, 2008.
13) Weinstock, N. et al.: *J. Thromb. Haemost.*, **4**: 1825-1827, 2006.
14) Hippala, S. T. et al.: *Blood Coagul. Fibrinolysis*, **6**: 743-746, 1995.

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—【原　著】— Original —

術中大量出血を防ぐための新たな輸血治療
—クリオプレシピテートおよびフィブリノゲン濃縮製剤投与効果の検討—

山本　晃士[1)]　西脇　公俊[2)]　加藤　千秋[1)]　花井　慶子[1)]　菊地　良介[1)]
柴山　修司[1)]　梛野　正人[3)]　木内　哲也[4)]　上田　裕一[5)]　髙松　純樹[1)]

＜背景・目的＞手術関連死亡の最大原因は術中の大量出血であるが，その背景には外科的手技による止血が不可能な希釈性凝固障害という病態が存在する．したがって術中の大量出血を未然に防ぐには止血のための輸血治療が必要であり，その治療指針の確立が急務である．＜方法・結果＞術中の大量出血・大量輸血症例を後方視的に調査した結果，その60％強を胸部大動脈瘤手術，肝臓移植術，肝臓癌・肝門部癌切除術が占めていた．術中大量出血の背景にある止血不全の主要因は，出血量の増加にともなう凝固因子（特にフィブリノゲン）の喪失，枯渇であると考えられた．そこで上記症例の手術中に起こった低フィブリノゲン血症に対し，クリオプレシピテートおよびフィブリノゲン濃縮製剤の投与を行ったところ，速やかなフィブリノゲン値の上昇と止血の改善，および術中出血量・輸血量の顕著な減少（平均で30～40％減）を認めた．＜結論＞術中の出血量増加時には，フィブリノゲン値を確認した上で速やかにフィブリノゲン濃縮製剤を投与することが，大量出血・大量輸血を未然に防ぎ，手術患者の予後改善に大きく貢献するとともに，血液製剤の使用削減・有効利用につながると考えられた．

キーワード：大量出血，希釈性凝固障害，低フィブリノゲン血症，胸部大動脈瘤，肝臓移植術

緒　言

手術関連死亡の最大の原因は術中の大量出血である．大量出血時には循環血液量の維持や赤血球輸血が重要であることは言うまでもないが，希釈性凝固障害が引き起こす止血不全に対する治療が大量出血を未然に防ぐ鍵となる[1)2)]．従来，術中の大量出血および止血不全に対しては新鮮凍結血漿および濃厚血小板製剤の投与が標準的な治療であったが，その効果は不十分であることが多く，止血のための真に有効な輸血治療の確立が急務である．本研究はまず術中大量出血時の止血不全の病態機序を解明するため，術中に大量出血をきたすことの多い基礎疾患・術式について後方視的な調査を行い，術中の止血・凝固能を詳細に解析した．その上で，新鮮凍結血漿投与に替わる新たな治療としてクリオプレシピテートもしくはフィブリノゲン濃縮製剤の投与を行い，凝固検査値および止血の改善度，術中の出血量・輸血量，患者の予後等に及ぼす影響を検討した．以上を通じて，術中大量出血時における止血のための輸血指針を提言したい．

方　法

まず術中の大量出血症例の実態を把握するため，当院において2005年1月から2006年12月までの2年間，術中に4,000m*l*以上の大量出血をきたした症例，および濃厚赤血球製剤20単位以上の大量輸血を行った症例のカルテ調査を行った．調査項目は，年齢・性別，疾患名，術式，術前状態，出血量，輸血量，術前・術中の血液凝固検査データ，生命予後などである．

次に，上記の検討によって明らかとなった術中大量出血をきたしやすい基礎疾患・術式について，術中出血量の増加時における凝固能の変化を解析し，止血不全に至る要因につき検討した．具体的には，胸部大動脈瘤症例，肝臓移植術症例，肝臓癌・肝門部癌症例において，循環血液量に相当するほどの大量出血をきたした場合，一定の時間間隔でヘモグロビン値および血小板数測定と血液凝固検査（PT，APTT，フィブリノ

1）名古屋大学医学部附属病院輸血部
2）名古屋大学医学部附属病院麻酔科
3）名古屋大学医学部附属病院腫瘍外科
4）名古屋大学医学部附属病院移植外科
5）名古屋大学医学部附属病院胸部外科
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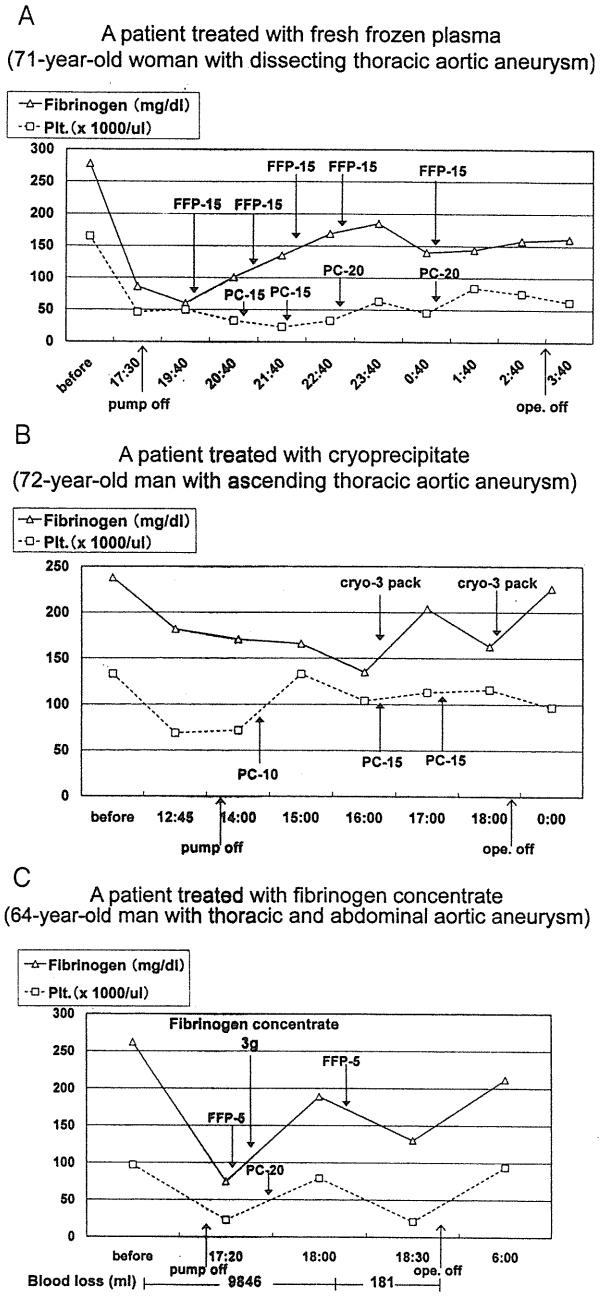

Fig. 1　Time course of fibrinogen levels and platelet counts during surgery in patients with thoracic aortic aneurysm. A: a case treated with fresh frozen plasma as hemostatic therapy; B: a case treated with cryoprecipitate (cryo); C: a case treated with fibrinogen concentrate. FFP: fresh frozen plasma (units); PC: platelet concentrate (units); Plt.: platelet counts; before: before operation; ope. off: the end of operation.

ゲン値の測定）を行った．止血のための輸血治療としてまず新鮮凍結血漿や濃厚血小板製剤の投与を行い，凝固検査値および実際の止血の改善度を評価した．そして止血に難渋するような全身性の出血傾向を認めた場合には，凝固検査値を確認した上でクリオプレシピテート製剤（当院輸血部にてあらかじめ新鮮凍結血漿5単位製剤より作製し−40℃で保存しておいたもの）を3パック（新鮮凍結血漿15単位分）もしくはフィブリノゲン濃縮製剤（フィブリノゲン-HT®）3gの投与を行い，その後の出血量と血小板数・血液凝固能（PT, APTT, フィブリノゲン値）の変化，および実際の止血の改善度について検討した．フィブリノゲン補充効果の高いこれらの製剤を投与することで，術中の大量出血を食い止めることができるかどうか，血液検査値の評価も含めて新鮮凍結血漿投与の場合との比較検討を行った．なお，肝臓移植術の一部症例では2005年8月より術中にフィブリノゲン濃縮製剤を使用している[3]．

2008年からは，胸部外科手術，肝臓移植術，肝臓癌・肝門部癌手術症例において術中の出血量増加時に血中フィブリノゲン値の即時評価を行い，低フィブリノゲン血症を認めた場合にはすみやかにフィブリノゲン濃縮製剤によるフィブリノゲン補充治療を積極的に行って，その止血効果を検討した．この新たな止血治療が各手術における出血量・輸血量の増減に及ぼす効果について，出血量に応じて迅速にフィブリノゲン値測定およびフィブリノゲン補充を行っていなかった2007年までの実態と比較検討した．

なお本研究の実施に際しては，手術中の止血治療として保険適応のないフィブリノゲン濃縮製剤を投与することについて院内倫理委員会の承認を得た．また，本研究の対象となる可能性のある手術予定の患者に対しては，あらかじめ大量出血が起こる可能性について説明し，その際の治療としてクリオプレシピテートもしくはフィブリノゲン濃縮製剤を投与することがある旨を話し，書面での同意を取得した．

結　果

2005年から2006年の2年間に83例の術中大量出血（4,000ml以上）もしくは大量輸血（赤血球製剤20単位以上）症例を認めたが，そのうち特に多かったのは胸部大動脈瘤に対する人工血管置換術（24例：29%），肝臓移植術（14例：17%），肝臓癌・肝門部癌切除術（14例：17%）の3つであり，次いで心臓弁膜症（9例：11%），産婦人科手術（3例），腎臓癌摘出術（2例）であった．手術症例の術前の血液凝固検査値を見てみると，胸部大動脈瘤症例では約3分の2の症例でFDPおよびD-dimerの軽度〜中等度上昇を認めた．また肝硬変を背景とする肝臓移植術，肝臓癌摘出術症例のほとんどで血小板数の低下，PTの延長（60% 未満）およびフィブリノゲン値の低下（150mg/dl以下）を認めた．しかし，止血目的の輸血治療の必要性を判断するのに必須な凝固検査が術中に行われていた症例はごく一部であり，一般的には行われていないことが明らかとなった．術中大量出血症例においては，フィブリノゲン値を術中