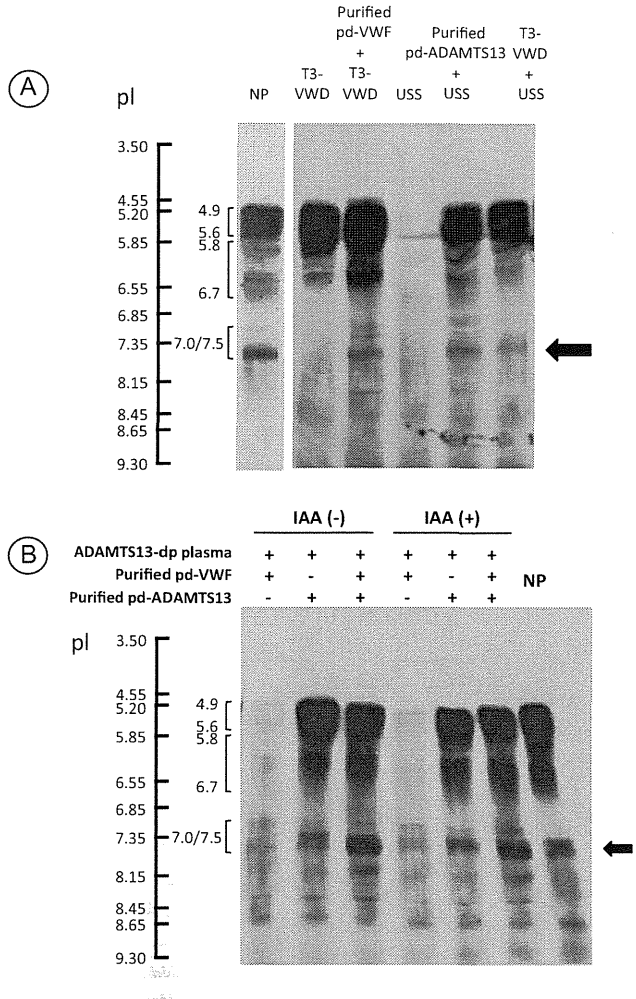

Fig. 2. Spiking experiments and effect of IAA treatment on VWF-ADAMTS13 complex formation. (A) VWF-ADAMTS13 complex formation was analyzed by IEF and immunoblotting with anti-ADAMTS13 MoAb (WH2-11-1). Plasma of T3-VWD spiked with the purified pd-VWF generated a new band at pI 7.5. When plasma from a patient with USS was spiked with purified pd-ADAMTS13, the band at pI 7.5 clearly appeared. When plasmas from T3-VWD and USS patients were mixed together, the band at pI 7.5 also appeared, confirming that it represents a complex of VWF and ADAMTS13. (B) Purified pd-VWF (3 μg), pd-ADAMTS13 (200 ng), and ADAMTS13-dp plasma were treated for 30 minutes at room temperature with 100 mmol/L IAA. ADAMTS13-dp plasma was mixed with purified pd-VWF (final concentration, 60 μg/mL) and/or pd-ADAMTS13 (final concentration, 2.3 μg/mL). Mixtures were exposed to high shear stress in a vortex mixer at maximum speed (3200 rpm) for 5 minutes. The VWF-ADAMTS13 complex, represented by the band at pI 7.5, is generated irrespective of the presence of IAA. Purified pd-VWF spiked into this mixture increased the density of this band. Arrow indicates the VWF-ADAMTS13 complex.

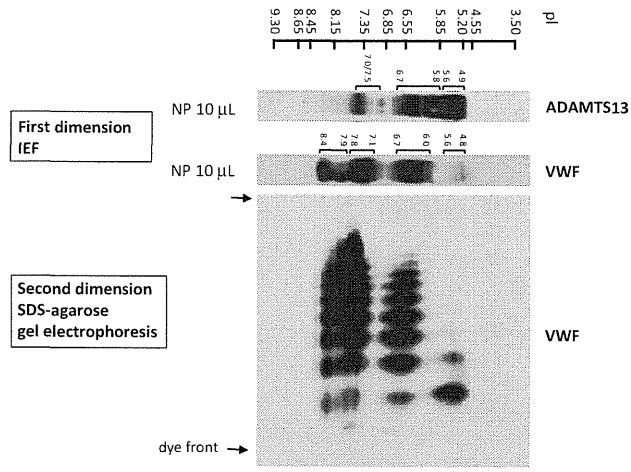

Fig. 3. Two-dimensional analysis of VWF in NP. Ten microliters of NP was subjected to IEF and immunoblotting with anti-ADAMTS13 MoAb (WH2-11-1; upper panel) and polyclonal anti-VWF antibody (middle panel). IEF gel of NP was subjected to a second dimension of electrophoresis on a SDS-0.9% agarose gel and immunoblotting with anti-VWF polyclonal antibody. In the lower panel, the upper arrowhead indicates the start point of two-dimensional SDS-0.9% agarose gel electrophoresis; the lower arrowhead indicates the position of the dye front at the termination of electrophoresis. VWF antigen in NP was separated into four series of pI bands (4.8-5.6, 6.0-6.7, 7.1-7.8, and 7.9-8.4) by IEF (middle panel), whereas ADAMTS13 antigen was separated into three series of pI bands (4.9-5.6, 5.8-6.7, and 7.0/7.5; upper panel). Two-dimensional analysis of normal plasma by IEF followed by SDS-0.9% agarose gel electrophoresis (lower panel) revealed that plasma ADAMTS13 is primarily in complex with larger VWF multimers and to a lesser extent with smaller VWF multimers.

5.8-6.7, and 7.0/7.5; also see Fig. 1), and VWF antigen was largely separated into four series of bands: pI 4.8 to 5.6 (trace), 6.0 to 6.7, 7.1 to 7.8, and 7.9 to 8.4 (Fig. 3, middle).

Since two ADAMTS13 bands with pI 4.9 to 5.6 and 5.8 to 6.7 were seen in T3-VWD plasma (Fig. 1), both bands appeared to be present in plasma irrespective of the presence of VWF. Further, two-dimensional analysis of normal plasma (IEF gel followed by SDS-0.9% agarose gel electrophoresis) confirmed that ADAMTS13 forms a complex with a larger VWFM with pI 7.1 to 7.8, but less likely with a smaller VWFM (dimers and tetramers) with pI 4.8 to 5.6 (Fig. 3, lower panel).

Amounts of ADAMTS13 and VWF in CP and CSP

Gill and colleagues[23] reported that the level of VWF antigen in plasmas from normal individuals with blood group O is significantly lower than that in plasmas with non-O blood groups. Further, Feys and colleagues[12] indicated that ADAMTS13 is bound to VWF with a stoichiometry of one ADAMTS13 molecule to 250 VWF

TABLE 1. ADAMTS13 activity and VWF antigen levels in plasma products*

Plasma products	All (n = 120)	Blood type A (n = 30)	Blood type O (n = 30)	Blood type B (n = 30)	Blood type AB (n = 30)	Overall p value
ADAMTS13 activity (%)						
FFP	81 ± 16	84 ± 17	77 ± 15	80 ± 16	83 ± 13	NS
CSP	71 ± 14	72 ± 14	69 ± 17	70 ± 12	72 ± 13	NS
Recovery (%)†	92.7 ± 3.7	92.9 ± 3.6	95.2 ± 2.1	91.4 ± 3.9	91.2 ± 3.5	<0.01[a]
CP	5.6 ± 2.8	5.4 ± 2.4	3.5 ± 1.6	6.5 ± 2.9	6.9 ± 2.8	<0.01[a]
Recovery (%)†	7.3 ± 3.7	7.1 ± 3.6	4.8 ± 2.1	8.6 ± 3.9	8.8 ± 3.5	<0.01[a]
VWF antigen (%)						
FFP	124 ± 46	121 ± 49	80 ± 24	144 ± 32	150 ± 38	<0.01[b]
CSP	16 ± 7	15 ± 7	11 ± 3	19 ± 5	19 ± 6	<0.01[c]
Recovery (%)†	14.0 ± 2.6	12.7 ± 2.5	14.6 ± 2.2	14.6 ± 2.9	14.2 ± 2.4	<0.05[d]
CP	98 ± 35	100 ± 37	64 ± 18	112 ± 22	116 ± 32	<0.01[e]
Recovery (%)†	86.0 ± 2.6	87.3 ± 2.5	85.4 ± 2.2	85.4 ± 2.9	85.8 ± 2.4	<0.05[d]

* Overall p values were calculated using the Kruskal-Wallis H test. Significant differences between four groups (overall $p < 0.05$) were further analyzed by Mann-Whitney U test with Bonferroni correction.
† Recovery was calculated as the level in CP or CSP divided by the total (sum of levels in CP and CSP).
[a] $p < 0.01$ between O and B, AB, <0.05 between O and A.
[b] $p < 0.01$ between O and A, B, AB, <0.05 between A and B.
[c] $p < 0.01$ between O and A, B, AB, <0.01 between A and B, <0.05 between A and AB.
[d] $p < 0.05$ between A and O, B, AB.
[e] $p < 0.01$ between O and A, B, AB.
NS = no significant difference ($p \geq 0.05$).

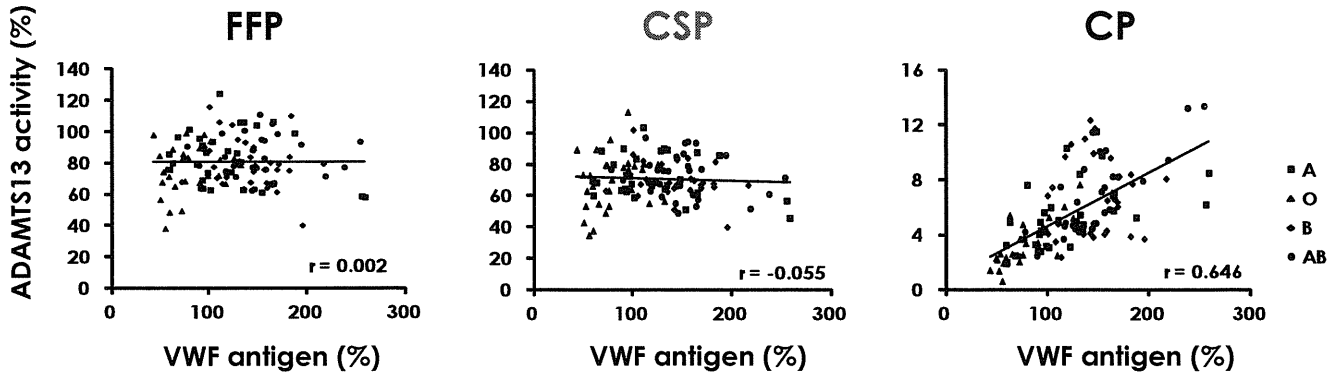

Fig. 4. Correlation between VWF antigen and ADAMTS13 activity in FFP, CSP, and CP. The correlation between ADAMTS13 activity and VWF antigen in FFP, CSP, and CP was analyzed. In FFP and CSP, there is no correlation between ADAMTS13 activity and VWF antigen. On the other hand, a significant correlation was observed between the two variables in CP ($r = 0.646$, $p < 0.01$).

monomeric subunits. Taking these two reports together, it is conceivable that the amount of a complex of ADAMTS13 and VWF in CP could be influenced by the ABO blood groups.

We analyzed ADAMTS13 activity and VWF antigen in FFP, CSP, and CP from 120 normal volunteers, with 30 individuals of each ABO blood type (A, B, O, and AB). The recovery rates of ADAMTS13 activity and VWF antigen in CP or CSP were expressed as the level in CP or CSP divided by the sum of the levels in both (CP + CSP). As summarized in Table 1, a mean of 7.3% (range, 4.8%-8.8%) of plasma ADAMTS13 activity was recovered in CP, whereas a mean of 92.7% (range, 91.2%-95.2%) remained in CSP. The amounts of ADAMTS13 remaining in CP from A, O, B, and AB plasmas were 5.4 ± 2.4, 3.5 ± 1.6, 6.5 ± 2.9, and 6.9 ± 2.8%, respectively; the amount of ADAMTS13 activity in CP was significantly lower in blood group O than in other blood groups. On the other hand, a mean of 86.0% (range, 85.4%-87.3%) of plasma VWF antigen was recovered in CP, whereas a mean of 14.0% (range, 12.7%-14.6%) remained in CSP. The amounts of VWF antigen in FFP, CP, and CSP from blood group O were significantly lower than in samples from other blood groups. The recovery rate of VWF antigen in CP was significantly higher in blood group A than in other blood groups.

As shown in Fig. 4, we analyzed the correlation between the levels of ADAMTS13 activity and VWF antigen in three plasma preparations with the Spearman rank test. We did not find a significant correlation between VWF antigen in FFP and ADAMTS13 activity in either FFP or CSP. In contrast, we did observe a significant correlation between ADAMTS13 activity in CP and VWF antigen in

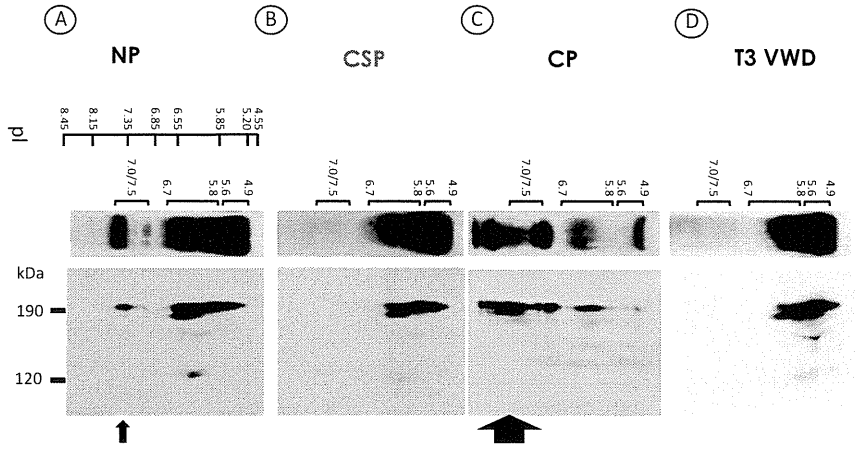

Fig. 5. ADAMTS13 in plasma fractions separated by IEF followed by SDS-PAGE. Normal plasma (NP), CSP, CP, and T3-VWD plasmas were subjected to IEF (upper panel). ADAMTS13 separated by IEF was subjected to a second dimension of electrophoresis by SDS-5% PAGE under reducing conditions and then to immunoblotting with anti-ADAMTS13 MoAb (WH2-11-1). (A) All three groups of ADAMTS13 bands (4.9-5.6, 5.8-6.7, and 7.0/7.5) in NP appeared as a 190-kDa by SDS-5% PAGE. Arrow indicates the VWF-ADAMTS13 complex. (B, D) The ADAMTS13 band at pI 7.0 or 7.5 was completely absent in CSP, almost indistinguishable to the case in T3-VWD plasma. (C) In CP, two faint bands with pI ranges of 4.9 to 5.6 and 5.8 to 6.7 and several strong bands with pI beyond 7.0 were detected. Arrow indicates the VWF-ADAMTS13 complex.

FFP ($r = 0.646$, $p < 0.01$; Fig. 4, right). These results indicate that the decreased level of ADAMTS13 activity in CP of blood group O was correlated with the low level of VWF antigen.

Cryoprecipitation efficiently removes the VWF-ADAMTS13 complex from plasma

To determine whether cryoprecipitation can remove the VWF-ADAMTS13 complex from plasma, we performed two-dimensional analysis (IEF followed by SDS-5% PAGE) under reducing conditions. As shown in Fig. 5A, all three groups of ADAMTS13 bands (pI 4.9-5.6, 5.8-6.7, and 7.0/7.5) in normal plasma migrated mainly as a 190-kDa band in SDS-5% PAGE, indicating that all three groups of bands included ADAMTS13. In CSP, however, the pI 7.0 or 7.5 band of ADAMTS13 was totally absent, almost indistinguishable from the case of T3-VWD plasma (Figs. 5B and 5D). Furthermore, when CSP was spiked with purified pd-VWF, a new band with pI 7.5 was generated (data not shown), indicating that ADAMTS13 in CSP can bind to higher molecular weight VWFMs and form a complex, as is the case in FFP.

In CP, we observed two faint bands with pI ranges of 4.9 to 5.6 and 5.8 to 6.7, and also several strong bands with pI greater than 7.0 (Fig. 5C).

Down regulation of H-SIPA with purified pd-ADAMTS13, CP, and CSP

In H-SIPA using a mixture of normal washed PLTs, ADAMTS13-dp plasma, and purified pd-VWF, maximum PLT aggregation (approx. 70% light transmittance) was achieved in the absence of ADAMTS13 (Fig. 6A). Under this condition, purified pd-ADAMTS13 spiked into the mixture inhibited H-SIPA in a dose-dependent manner at ranges of 5% to 20% of ADAMTS13 activity, but this effect reached a plateau (approx. 20% light transmittance) at the ranges from 50% to 500% of ADAMTS13 activity (Fig. 6A). Addition of NMC-4 almost totally blocked the PLT aggregation (approx. 3% light transmittance).

Further, ADAMTS13 in both FFP and CSP from normal individuals inhibited H-SIPA in a dose-dependent manner at the ranges of 5% to 20% of ADAMTS13 activity (Figs. 6B and 6C), comparable to the effect of purified pd-ADAMTS13.

On the other hand, ADAMTS13 in CP did not clearly inhibit H-SIPA at the initial phase before 140 seconds, even at 20% of ADAMTS13 activity. However, at the later phase of H-SIPA, the aggregation curves were uniformly reversed at a final concentration of 5% to 20% of ADAMTS13 activity. Consequently, the maximum PLT aggregation at the endpoint at 340 seconds was almost indistinguishable from that of FFP or CSP. Thus, the inhibition rates (%) in CP at two time points (140 and 340 sec) with two different final concentrations (5 and 20%) of ADAMTS13 activity were measured in each three times at the same occasion, and the results were the following: $20.5 \pm 14.0\%$ (at 140 sec) versus $46.9 \pm 11.3\%$ (at 340 sec; $p = 0.012$) in the presence of 5% ADAMTS13 activity and $57.7 \pm 5.9\%$ (at 140 sec) versus $85.7 \pm 2.7\%$ (at 340 sec; $p = 0.004$) in the presence of 20% ADAMTS13 activity (figure not shown).

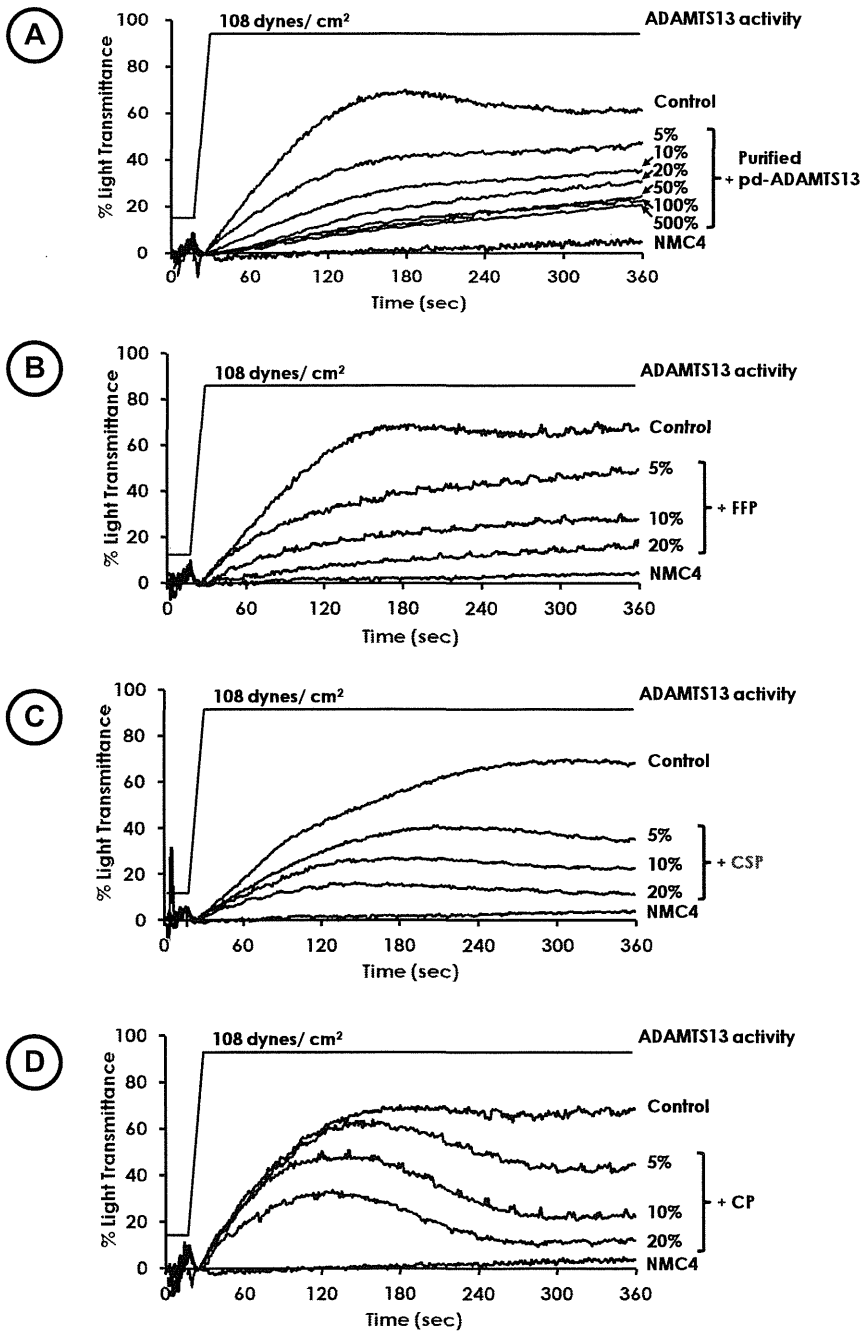

Fig. 6. Inhibitory effect of ADAMTS13 on H-SIPA. (A) The purified pd-ADAMTS13 inhibits H-SIPA in a dose-dependent manner at ranges of 5% to 20% of ADAMTS13 activity in the mixture. The inhibition reaches a plateau (approx. 20% light transmittance) at ranges of 50% to 500% of ADAMTS13 activity. (B, C) ADAMTS13 in both FFP and CSP from normal individuals exhibited dose-dependent inhibition of H-SIPA at the ranges of 5% to 20% of ADAMTS13 activity. (D) ADAMTS13 in CP did not clearly inhibit H-SIPA at the initial phase at less than 10% of ADAMTS13 activity. The inhibition of PLT aggregation was found at the ranges of 5% to 20% of ADAMTS13 activity; at the later phase of H-SIPA the maximum aggregation at the endpoint was almost the same as in FFP and CSP.

DISCUSSION

Using IEF analysis with a large-pore agarose-acrylamide composite gel, we have shown that ADAMTS13 in the plasma milieu is present in a complex with larger VWFMs, but is less likely to form complexes with smaller VWFMs (dimers and tetramers). Thus, cryoprecipitation followed by centrifugation could efficiently separate the two forms of ADAMTS13, with the VWF-bound ADAMTS13 in CP and the free ADAMTS13 and ADAMTS13 bound to smaller VWFMs in CSP. In support of these results regarding coprecipitation of ADAMTS13 and VWF, the ADAMTS13 activity levels we observed were closely correlated with VWF antigen levels in CP ($r = 0.646$), but not in either CSP ($r = -0.055$) or FFP ($r = 0.002$; Fig. 4). In addition, we observed no difference among blood groups with respect to the recovery rate of VWF antigen in CP, but the VWF antigen level in CP was lower in blood group O than in the other blood groups. As a result, both the ADAMTS13 activity and the VWF antigen levels in CP were significantly lower in blood group O than in non-O blood groups (Table 1). Further, we determined that approximately 95% of the original ADAMTS13 activity in FFP is recovered after cryoprecipitation; approximately 93% of the recovered ADAMTS13 activity remained in CSP, whereas 7% was found in CP. This relative distribution of ADAMTS13 in FFP and CSP was consistent with previous reports.[24-26]

Evidence that the pI 7.0 or 7.5 band is a complex of VWF and ADAMTS13 is clearly provided by the following observations: 1) plasmas from both VWF antigen–defective T3-VWD and ADAMTS13 antigen–defective USS patients lacked the bands at pI 7.0 or 7.5; 2) an equal mixture of plasmas from T3-VWD and USS generated the bands at pI 7.0 or 7.5; and 3) CSP prepared from normal plasma lacked the bands at pI 7.0 or 7.5, whereas CSP spiked with purified VWF regenerated these bands. On the other hand, we assume that the proteins in the two other band groups (pI 4.9-5.6 and 5.8-6.7) are less involved

in complex formation with VWF, because both band groups are present in T3-VWD plasma. Furthermore, because pd-ADAMTS13 purified from pooled normal plasmas has only one band with pI 4.9 to 5.6,[14] the pI 5.8 to 6.7 band might represent a complex with proteins other than VWF. This speculation originates from the observation that ADAMTS13 can bind in vitro to a soluble form of CD36[27] and Lys-plasminogen.[28]

In a previous study, immunoprecipitation method using anti-VWF was used to show that approximately 3% of the total in plasma ADAMTS13 is bound to VWF.[12] By contrast, in our IEF gel analysis, coupled with densitometry, we observed that the amount of VWF-bound ADAMTS13 in plasma appeared to be much lower than the amount of unbound ADAMTS13, but greater than the 3% of total ADAMTS13 (Fig. 1).[12] This discrepancy might be attributable to differences in the experimental designs employed in these studies.

The mechanism by which ADAMTS13 binds to VWF in the plasma milieu is a critical issue that remains to be addressed. Because Fujikawa and coworkers[29] succeeded in purifying ADAMTS13 from a commercial concentrate of Factor VIII and VWF, prepared from CP, such concentrates might contain the VWF-ADAMTS13 complex itself. After extensive fractionation, including fibrin-clot formation, ammonium sulfate precipitation, and sequential chromatography, the purified ADAMTS13 described in that study was free of VWF. However, in our experience, the VWF-ADAMTS13 complex in CP is not readily dissociated by size-exclusion chromatography in the presence of either 0.15 or 1 mol/L NaCl (data not shown). In fact, when we treated CP with 1 mol/L NaCl for 1 hour at room temperature before IEF, the bands at pI 7.0 or 7.5 persisted, indicating that no dissociation of VWF-ADAMTS13 complex had taken place under conditions of high ionic strength. These results may indicate that VWF binding to ADAMTS13 is independent of ionic strength. In this regard, McKinnon and colleagues[30] reported that the N-linked glycans of VWF exert a modulatory effect on the interaction with ADAMTS13 and that removal of the N-linked glycans from VWF increased its affinity for ADAMTS13 under static conditions. Furthermore, Yeh and coworkers[19] recently reported that ADAMTS13 possesses a disulfide bond–reducing activity that regulates shear-induced thiol-disulfide exchange. Therefore, one of the mechanisms underlying formation of VWF-ADAMTS13 complexes might involve disulfide-bond formation between ADAMTS13 and VWF. To address this issue, we investigated whether IAA, a blocker of free thiols, might prevent the formation of a disulfide bond–mediated covalent complex under high shear stress. We observed, however, that VWF-ADAMTS13 complex formation was unaffected by IAA treatment, suggesting that in CP, the amount of VWF-ADAMTS13 complex formed in a thiol-dependent fashion is marginal. This finding rules out a major role for disulfide bonds, but otherwise we have not elucidated the binding mechanism of VWF and ADAMTS13; this issue remains to be addressed in future studies.

PE is a first-line treatment for acquired TTP. For this purpose, either FFP or CSP is commonly used,[31] but the results regarding CP have been controversial.[32] At least one case of congenital TTP (USS) has been successfully treated with CP.[9] Therefore, it is important to determine whether there is a functional difference between bound and unbound ADAMTS13 and whether any such difference has physiologic relevance. Here we have clearly shown that CSP contains the unbound or less bound ADAMTS13, whereas CP contains much more bound ADAMTS13 and lower levels of its unbound counterpart. An authoritative determination regarding which form of ADAMTS13 more efficiently down regulates H-SIPA will be crucial in establishing the optimal treatment modality for TTP patients.

In most acquired TTP patients, plasma ADAMTS13 activity is less than 5% of normal. As a consequence, UL-VWFMs are not cleaved after secretion from endothelial cells and remain anchored to the cell surface in long strings.[33] Circulating PLTs adhere to these long strings, resulting in occlusive PLT thrombi. However, smaller VWFMs do not induce this spontaneous adhesion and aggregation of PLTs. Consequently, increased fluid shear stress is required to induce PLT aggregation in vitro.[2]

To reproduce the PLT aggregation generated in the microvasculature of ADAMTS13 activity–deficient TTP patients, here we employed an H-SIPA assay system that uses a mixture of washed normal PLTs and ADAMTS13-dp plasma spiked with purified pd-VWF to mimic TTP plasmas. In this assay, the purified pd-ADAMTS13 inhibited H-SIPA in a dose-dependent manner, reaching a plateau of 20% ADAMTS13 activity at final pd-ADAMTS13 concentrations up to 500%. Under the same experimental conditions, FFP, CSP, and CP inhibited H-SIPA in a dose-dependent manner to the same extent at the end points; in CP, however, the aggregation inhibition curves were different, and in fact no distinct inhibition was observed at the initial phase of PLT aggregation. This might be simply explained by the fact that the VWF concentration in the H-SIPA reaction mixtures was much higher than in CSP or FFP. Alternatively, the binary complex of ADAMTS13 and larger VWFMs might modulate a different phase of H-SIPA than unbound ADAMTS13, because CSP spiked with purified VWF readily generates the ADAMTS13–larger VWFM complex with pI 7.0 or 7.5. The larger VWFM is required in the earliest phase of PLT thrombi formation and high shear stress, but in the later phase the ADAMTS13–larger VWF complex embedded in the thrombi may play a role in regulating the size of the thrombi to prevent microvascular occlusion. Further studies are required to determine the functional differences between ADAMTS13 in CSP and CP.

In conclusion, our results indicated that both plasma products of FFP and CSP are effective in treatment of TTP. However, CSP may be more favorable for PE in acquired TTP patients: relative to FFP, CSP has a lower level of VWF and a comparable ADAMTS13 activity, but lower amounts of ADAMTS13–larger VWFM complex.

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CONFLICT OF INTEREST

YH, MH, AI, and KS have no conflict of interest. MM is a member of the clinical advisory board for Alexion Pharma. YF is a member of the clinical advisory boards for Baxter BioScience and Alexion Pharma.

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RATIO OF VON WILLEBRAND FACTOR PROPEPTIDE TO ADAMTS13 IS ASSOCIATED WITH SEVERITY OF SEPSIS

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ABSTRACT—Von Willebrand factor (VWF)–cleaving protease (ADAMTS13) cleaves ultralarge VWF (ULVWF) secreted from endothelium and by which is regulating its physiologic function. An imbalance between ULVWF secretion and ADAMTS13 level occurs in sepsis and may cause multiple organ dysfunction. We evaluated the association between the VWF-propeptide (VWF-pp)/ADAMTS13 ratio and disease severity in patients with severe sepsis or septic shock. In 27 patients with severe sepsis or septic shock and platelet count less than 120 000/μL, we measured plasma VWF, VWF-pp, and ADAMTS13 levels on hospital days 1, 3, 5, and 7. The VWF-pp/ADAMTS13 ratio was increased greater than 12-fold in patients with severe sepsis or septic shock on day 1 and remained markedly high on days 3, 5, and 7 compared with normal control subjects. The VWF-pp/ADAMTS13 ratio significantly correlated with Acute Physiology and Chronic Health Evaluation II score on days 1 and 5; Sepsis-related Organ Failure Assessment score on days 1, 3, and 5; maximum Sepsis-related Organ Failure Assessment score and tumor necrosis factor α level on days 1, 3, 5, and 7; and creatinine level on days 1, 5, and 7. Patients with greater than stage 1 acute kidney injury had significantly higher VWF-pp/ADAMTS13 ratio than patients without acute kidney injury. In summary, the VWF-pp/ADAMTS13 ratio was associated with disease severity in patients with severe sepsis or septic shock and may help identify patients at risk for multiple organ dysfunction by detecting severe imbalance between ULVWF secretion and ADAMTS13 level.

KEYWORDS—Sepsis, von Willebrand factor propeptide, ADAMTS13, multiple organ dysfunction

INTRODUCTION

Severe sepsis and septic shock result from the systemic host response to infection, including inflammation, coagulation, and changes in the vascular endothelium. Vascular endothelial activation, dysfunction, and injury facilitate leukocyte and platelet aggregation and aggravate inflammation and thrombosis (1). Von Willebrand factor (VWF) is a key marker of endothelial changes (2).

Von Willebrand factor is a multimeric glycoprotein that circulates in plasma and functions as a bridge between the subendothelial matrix and platelets. The subunit precursor proVWF (350 kd) is synthesized in the endothelium and contains signal peptide, VWF propeptide (VWF-pp), and VWF subunit. The proVWF is dimerized through disulfide bonds after removal of signal peptide in the endoplasmic reticulum. The proVWF dimers are transported to the Golgi apparatus, VWF-pp is cleaved, and additional disulfide bonds form between proVWF dimers to yield ultralarge VWF (ULVWF; size, >20,000 kd). Ultralarge VWF condenses into tubules and forms Weibel-Palade bodies. Ultralarge VWF and VWF-pp are stored in Weibel-Palade bodies in equimolar amounts on a subunit basis (3, 4).

Several inflammatory mediators, such as thrombin, histamine, and proinflammatory cytokines, including tumor necrosis factor α (TNF-α) and interleukin 8 (IL-8), activate endothelial cells and induce Weibel-Palade body exocytosis (5, 6), causing cell surface expression of ULVWF and release of VWF-pp into the bloodstream. Because longer VWF is more active, and ULVWF causes spontaneous platelet aggregation and thrombosis, it is immediately cleaved by VWF cleaving protease after secretion, which is also known as a disintegrin-like and metalloprotease with thrombospondin type 1 motif, member 13 (ADAMTS13). This cleavage results in smaller and less adhesive plasma forms of VWF (7). In the absence of ADAMTS13, secreted ULVWF strings that are bound to endothelium are not cleaved but adhere to platelets, which bind to leukocytes and cause thrombosis and inflammation (8, 9).

Because an appearance of ULVWF in plasma has been demonstrated in patients with inadequate function of ADAMTS13, as in thrombotic thrombocytopenic purpura (TTP) or sepsis (10, 11), it may suggest an imbalance between ULVWF secretion and ADAMTS13 function. Plasma ULVWF may be a good marker to detect this imbalance, but it is technically difficult to determine ULVWF and quantify it. Furthermore, plasma ULVWF often cannot be detected in patients having

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this imbalance and developing organ failure, as in chronic relapsing TTP (12).

The mean or median levels of ADAMTS13 are decreased to 20% to 43% normal in sepsis (13–15). However, ADAMTS13 level less than 10% normal is enough to prevent the clinical manifestation of primary thrombotic microangiopathy in patients with congenital ADAMTS13 deficiency (16). This suggests that patients with sepsis have a high enough ADAMTS13 level to prevent thrombotic microangiopathy, but it may not be high enough to cleave all ULVWF secreted from endothelium during sepsis. Furthermore, multiple organ dysfunction in children with thrombocytopenia was resolved by restoring ADAMTS13 activity by plasma exchange (17). Therefore, both decreased ADAMTS13 level and the imbalance between ULVWF secretion and ADAMTS13 activity may cause microvascular thrombosis formation in sepsis. If so, it may be clinically relevant to measure the imbalance between ULVWF secretion and ADAMTS13 activity.

The VWF-pp is secreted in equimolar amounts to the total subunits of secreted ULVWF and more rapidly cleared from the circulation than VWF (half-life: VWF-pp, 3 h; VWF, 12 h) (5). Therefore, we hypothesized that VWF-pp level may reflect ULVWF secretion and that the VWF-pp/ADAMTS13 ratio may be a sensitive and real-time measure of imbalance between ULVWF secretion and plasma ADAMTS13 level. Higher VWF-pp/ADAMTS13 ratio may reflect insufficient control of VWF multimer size, and this may accelerate microvascular thrombus formation, inflammation, and organ failure.

The purpose of this study was to investigate whether the VWF-pp/ADAMTS13 ratio is associated with disease severity in patients with severe sepsis or septic shock. Although there have been several previous studies about VWF, VWF-pp, and ADAMTS13 levels in patients with severe sepsis or septic shock, limited information is available about the time course of these levels simultaneously measured. We determined the time course of the levels of VWF, VWF-pp, and ADAMTS13 during the early phase of sepsis.

MATERIALS AND METHODS

Patients

From January 2008 to December 2009, all patients treated at the intensive care unit of the Department of Emergency and Critical Care Medicine, Nara Medical University Hospital, was considered for the study. Inclusion criteria for the study were (i) severe sepsis or septic shock as defined by published guidelines (18) and (ii) platelet count less than 120 000/μL. Exclusion criteria were (i) patients younger than 18 years, (ii) pregnancy, (iii) medical history of chronic renal failure (stage 5 chronic kidney disease) (19) or chronic liver disease (20), (iv) cardiopulmonary arrest, (v) other hematologic disorders that may lower the platelet count such as TTP, and (vi) malignancy. There were 27 patients included in the study. This study protocol was approved by the institutional review board of Nara Medical University Hospital. Written informed consent was obtained from enrolled patients or family members.

Evaluation

Clinical information was collected including age, sex, diagnosis, serum creatinine level, and survival status at 28 days after admission. Survivors were defined as patients who were alive 28 days after admission, and nonsurvivors were patients who died within 28 days after admission. The severity of disease and organ failure were assessed with Acute Physiology and Chronic Health Evaluation II (APACHE II) score (21) and Sepsis-related Organ Failure Assessment (SOFA) score (22) at days 1 (on admission), 3, 5, and 7 after admission. Maximum SOFA (Max SOFA) score was defined as the Max SOFA score during the clinical course at any time on day 28 or less. Acute kidney injury (AKI) stage was assessed by the criteria of the Acute Kidney Injury Network Working Group (23).

Assays

Citrated blood samples were obtained from patients who met the inclusion criteria on admission to the intensive care unit (day 1) and days 3, 5, and 7. Blood samples were centrifuged at 1,500*g* for 10 min in a cooled centrifuge immediately after drawing, and aliquots of plasma were stored at −80°C until assayed. Blood samples were obtained from 15 healthy volunteers (nine men and six women; age range, 23–55 years [mean, 40 years]) and pooled for ADAMTS13, VWF-pp, and VWF assays as the normal controls being 100%.

Activity of ADAMTS13 was assayed using a commercial kit (Kainos Laboratories, Inc, Tokyo, Japan). The plasma level of VWF-pp was measured with an enzyme-linked immunosorbent assay kit (Sanquin, Amsterdam, the Netherlands). Levels of IL-6 (R&D Systems Inc, Minneapolis, Minn), TNF-α (R&D Systems Inc), and VWF (Dako, Glostrup, Denmark) were measured.

Data analysis

Data analysis was performed with statistical software (SPSS, Inc, Armonk, NY; and GraphPad, San Diego, Calif). Data are reported as mean ± SD or median with interquartile range. The Shapiro-Wilk test was used to evaluate normality of data. Groups were compared with *t* test or Mann-Whitney *U* test, and the relation between two variables was evaluated with Spearman rank correlation. Statistical significance was defined by $P \leq 0.05$ for 2-sided tests.

RESULTS

Most patients were men, and the most common diagnosis was intra-abdominal infection (Table 1). Most patients (20 of 27 patients) were survivors; one patient with acute abdomen died on day 6, and the other 26 patients completed blood collection until day 7. All measurements did not differ between male and female patients. There were no differences between survivors and nonsurvivors in APACHE II score, SOFA score, serum creatinine level, platelet count, and fibrin degradation product level (data not shown).

In patients with severe sepsis and septic shock, the mean VWF level was high on day 1, and there was no significant change in mean VWF level from day 1 to day 7 (Table 2). The VWF level did not differ between survivors and nonsurvivors (data not shown). The level of VWF did not correlate with any clinical scores or laboratory markers (data not shown).

The mean VWF-pp level was high on day 1, remained high but decreased significantly from day 1 to day 3, and remained high from day 3 to day 7 (Table 2). There were no differences in mean VWF-pp level between survivors and nonsurvivors (data not shown). The levels of VWF-pp were correlated significantly with SOFA score on days 1, 3, and 5; with Max SOFA at days 5 and 7; and with TNF-α level on day 1 (Table 3).

The mean level of ADAMTS13 was significantly lower in patients on day 1 than normal controls, and the mean level of ADAMTS13 increased in patients significantly from day 1 to day 3 and from day 3 to day 5 (no difference between values on days 5 and 7) (Table 2). The mean level of ADAMTS13 was significantly higher in survivors than in nonsurvivors on days 1, 5, and 7 but not on day 3 (Table 2). The levels of ADAMTS13 correlated negatively with APACHE II score on days 1 and 5; SOFA score on day 5; Max SOFA score on days 1, 3, and 5; TNF-α on day 5; and IL-6 and creatinine levels on day 7 (Table 3).

The mean VWF-pp/ADAMTS13 ratio was 12-fold greater in patients on day 1 than normal control subjects, and the mean ratio decreased significantly in patients from day 1 to day 3

TABLE 1. **Clinical and laboratory findings on admission in patients with severe sepsis or septic shock***

	Total	Male (n = 16)	Female (n = 11)
Age, y	70 ± 16	71± 14	68 ± 19
APACHE-II score	21.0 ± 7.3	22.0 ± 7.4	24.0 ± 4.6
SOFA score	11.1 ± 3.3	12.3 ± 3.2	11.3 ± 2.8
SIRS score†	20	12	8
Survivors	20 (74)	13 (81)	7 (64)
Diagnosis			
Intra-abdominal infection	18 (67)	11 (69)	7 (64)
Urinary tract infection	3 (11)	1 (6.3)	2 (18)
Pneumonia	2 (7)	2 (13)	0
Burn wound sepsis	2 (7)	0	2 (18)
Necrotizing fasciitis	1 (4)	1 (6.3)	0
Descending mediastinitis	1 (4)	1 (6.3)	0
Acute Kidney Injury > stage 1	19 (70)	11 (69)	8 (73)
Platelet count, /μL	8.3 ± 3.0	9.1 ± 3.3	7.2 ± 2.2
Creatinine, mg/dL	1.7 ± 1.0	1.9 ± 1.0	1.4 ± 1.0
ADAMTS13, %	24.9 ± 8.5	25.9 ± 9.5	23.5 ± 7.4
von Willebrand factor propeptide, %	293.8 ± 153.8	294.6 ± 142.8	292.7 ± 175.7
von Willebrand factor, %	212.3 ± 86.3	225.2 ± 81.4	194.7 ± 83.8

ADAMTS13, von Willebrand factor propeptide, and von Willebrand factor are expressed as a percentage of normal controls. Data are reported as mean ± SD or number (%).
*n = 27 patients.
†Systemic inflammatory response syndrome score >3.

and remained markedly increased compared with controls at days 5 and 7 (Table 2). The VWF-pp/ADAMTS13 ratio correlated significantly with APACHE II score on days 1 and 5; with SOFA score on days 1, 3, and 5; and with Max SOFA score and TNF-α level on days 1, 3, 5, and 7 (Table 3). The IL-6 and TNF-α levels in patients on days 1 and 3 were markedly greater than the upper limit of normal (Table 2).

Nineteen patients with severe sepsis or septic shock developed AKI of greater than stage 1 within 48 h after admission (Table 1). The mean levels of VWF and ADAMTS13 did not differ between patients with or without AKI, but patients with AKI had significantly greater mean levels of VWF-pp (AKI, 338% ± 143%; no AKI, 190% ± 134%; $P \leq 0.02$) and VWF-pp/ADAMTS13 ratio (AKI, 15% ± 7%; no AKI, 7% ± 6%; $P \leq 0.001$) on day 1. The VWF-pp level correlated significantly with serum creatinine level on day 1, and the VWF-pp/ADAMTS13 ratio correlated significantly with serum creatinine level on days 1, 5, and 7 (Table 3).

DISCUSSION

A decreased level of ADAMTS13 on admission had been described previously in patients with sepsis (24) and correlated with AKI (11), APACHE II score, and poor prognosis (13). The present results confirmed that decreased ADAMTS13 levels correlated with disease severity scores including APACHE II and Max SOFA on the same days of observation including the day on admission (Table 3). The finding that means ADAMTS13 level was significantly lower in nonsurvivors than survivors on days 1, 5, and 7 (Table 2) suggests that ADAMTS13 level may be a prognostic marker for survival during the early phase of sepsis.

The cause of the decreased ADAMTS13 levels in sepsis is controversial. Possible mechanisms for the decrease include consumption because of excess substrate and proteolytic degradation by thrombin, plasmin, and neutrophil protease (11, 25). In addition, infusion of endotoxin or desmopressin into healthy volunteers may increase plasma VWF and VWF-pp levels and may decrease ADAMTS13 activity (26, 27); this suggests that ADAMTS13 may be consumed mainly by excessive ULVWF released by endotoxin or desmopressin, or secretion of ADAMTS13 may be inhibited. Greater duration or intensity of stimulation to endothelium, causing ULVWF secretion with proinflammatory cytokines such as TNF-α (28), may induce greater imbalance between ULVWF secretion and plasma ADAMTS13 level, resulting in larger VWF molecules in plasma and a prothrombotic condition.

What can be used to estimate the extent of the imbalance between ULVWF secretion and plasma ADAMTS13 level? The appearance of ULVWF in plasma may be a good marker for the imbalance between ULVWF secretion and plasma ADAMTS13 level (14). However, ULVWF can be detected only by time-consuming immunoblotting after electrophoresis, and it is difficult to quantify ULVWF reproducibly (11, 15). Furthermore, ULVWF is very adhesive to platelets and can cause spontaneous platelet aggregation, associated consumption, and decreased levels of ULVWF. The disappearance of ULVWF may be observed in some patients with chronic TTP during acute episodes (12). In addition, some studies show no correlation between ULVWF and decreased levels of ADAMTS13 (11, 29).

The ratio of VWF level to ADAMTS13 activity is reported to be more useful than VWF multimer analysis (ULVWF detection) alone for the diagnosis of highly prothrombotic states induced by the imbalance between VWF secretion and ADAMTS13 (15). However, plasma VWF level may not reflect ULVWF secretion accurately because VWF may be affected by ABO blood group antigens; in addition, secreted plasma VWF can be consumed at the endothelial injury site, especially during inflammation, by binding to the subendothelial matrix, endothelium, platelets, or white blood cells (9). An increased plasma level of VWF on admission is reported to be associated with an increased risk of death from severe sepsis (30); yet, the present study showed that markedly increased VWF levels in patients with severe sepsis or septic shock were not associated with disease severity during the first 7 days and showed increasing tendency despite resolution of clinical symptoms, consistent with other studies (15, 24). Thus, plasma VWF level did not likely reflect ULVWF secretion rate in the present study.

In contrast with VWF, the VWF-pp is not affected by ABO antigen and does not bind to the vascular wall; consequently, plasma level of VWF-pp may more accurately reflect ULVWF

TABLE 2. **Levels of VWF, VWF-pp, ADAMTS13, and inflammatory markers in patients with severe sepsis or septic shock***

		Patients with severe sepsis or septic shock			
		Day			
Variables	Control subjects	1	3	5	7
VWF, %	96 ± 14	212 ± 86	228 ± 85	240 ± 85	252 ± 112
VWF-pp, %	96 ± 16	294 ± 154	240 ± 115†	219 ± 117	228 ± 162
ADAMTS13, %					
All patients	100 ± 10	25 ± 8.5‡	30 ± 9‡	33 ± 11‡	33 ± 11
Survivors	NA	27 ± 8.6	31 ± 8.7	35 ± 9.4	36 ± 10
Nonsurvivors	NA	19 ± 5.4	27 ± 8.2	25 ± 10	24 ± 9.4
P	NA	0.03§	NS	0.03§	0.02§
VWF-pp/ADAMTS13 ratio	0.97 ± 0.18	12.9 ± 7.2‖	8.9 ± 5.1‖	7.7 ± 6.0	7.9 ± 7.1
IL-6,¶ pg/mL	<2.4	1,220 (362–3,610)	206 (58–1,050)	115 (29–338)	75 (20–446)
TNF α (pg/mL)**	<1.8	5.8 (3.3–21.3)	3.4 (2.4–5.8)	2.5 (1.2–4.0)	2.0 (1.4–3.3)

Data are reported as mean ± SD or median (interquartile range). VWF, VWF-pp, and ADAMTS13 are expressed as a percentage of normal controls.
*n = 27 patients (day 1) or 26 patients (days 3, 5, and 7) with sepsis or septic shock; 15 normal control subjects.
†VWF-pp: difference between days 1 and 3, $P \leq 0.05$.
‡ADAMTS13: difference between normal controls and patients on day 1, $P \leq 0.001$; difference between days 1 and 3, $P \leq 0.01$; difference between days 3 and 5, $P \leq 0.05$.
§ADAMTS13: difference between survivors and nonsurvivors, $P \leq 0.05$.
‖VWF-pp/ADAMTS13 ratio: difference between normal controls and patients on day 1, $P \leq 0.001$; difference between days 1 and 3, $P \leq 0.01$.
¶Upper limit of normal, 2.41 pg/mL.
**Upper limit of normal, 1.79 pg/mL.
NS indicates not significant ($P > 0.05$); NA, not applicable.

secretion induced by endothelial activation than VWF (5). In the present study, increased plasma VWF-pp level was associated with SOFA score and TNF-α on day 1 (Table 3), suggesting that VWF-pp may be a better marker of acute endothelial activation than VWF in the early phase of sepsis. The marked increase in VWF-pp level on admission significantly

TABLE 3. **Relation between VWF-pp, ADAMTS13, and clinical scores and markers in patients with severe sepsis or septic shock**

	Day 1		Day 3		Day 5		Day 7	
	*r**	*P* ≤	*r**	*P* ≤	*r**	*P* ≤	*r**	*P* ≤
VWF-pp								
APACHE II	0.32	NS	0.16	NS	0.45	0.05	0.07	NS
SOFA	0.51	0.007	0.47	0.02	0.55	0.01	−0.62	NS
Max SOFA	0.25	NS	0.38	NS	0.44	0.03	0.59	0.003
TNF-α	0.47	0.02	0.54	NS	0.38	NS	0.44	NS
IL-6	0.20	NS	−0.11	NS	0.25	NS	0.42	NS
Creatinine	0.59	0.001	0.34	NS	0.32	NS	0.18	NS
ADAMTS-13								
APACHE II	−0.54	0.004	−0.30	NS	−0.68	0.001	−0.34	NS
SOFA	−0.32	NS	−0.30	NS	−0.57	0.007	−0.13	NS
Max SOFA	−0.53	0.005	−0.42	0.03	−0.47	0.02	−0.29	NS
TNF-α	−0.07	NS	−0.37	NS	−0.40	0.05	−0.43	NS
IL-6	−0.04	NS	−0.36	NS	−0.39	NS	−0.45	0.05
Creatinine	−0.33	NS	−0.22	NS	−0.35	NS	−0.47	0.02
VWF-pp/ADAMTS-13 ratio								
APACHE II	0.45	0.03	0.15	NS	0.69	0.001	0.19	NS
SOFA	0.65	0.001	0.41	0.04	0.68	0.001	−0.61	NS
Max SOFA	0.45	0.03	0.41	0.04	0.52	0.007	0.63	0.001
TNF-α	0.44	0.03	0.57	0.002	0.44	0.03	0.59	0.007
IL-6	0.12	NS	0.04	NS	0.48	0.02	0.70	0.001
Creatinine	0.76	0.001	0.29	NS	0.49	0.02	0.48	0.02

*Spearman rank correlation (ρ).
NS indicates not significant ($P > 0.05$).

decreased by day 3, but remained more than 2-fold greater than normal for at least 7 days (Table 2), and this is evidence of persistent endothelial activation in sepsis. This also is consistent with previous studies that showed increased plasma VWF-pp level in sepsis and association with SOFA score and creatinine level but not with prognosis (24, 31).

The VWF-pp/ADAMTS13 ratio significantly correlated with disease severity including APACHE II score, SOFA score, the proinflammatory cytokine TNF-α, and creatinine during the period of observation (Table 3). Marked increase in the VWF-pp/ADAMTS13 ratio seemed to correlate with disease severity better than VWF-pp or ADAMTS13 level alone in patients with severe sepsis or septic shock. These results suggest that an imbalance between ULVWF secretion and ADAMTS13 level induced by endothelial activation or dysfunction may cause microthrombi and inflammation that lead to organ failure. In a porcine model of *Escherichia coli* sepsis, observations included decreased ADAMTS13 level, increased proportion of large-molecular-weight VWF multimers, glomerular microthrombi enriched with platelets and VWF, and acute renal failure (28). Therefore, the imbalance between VWF secretion and ADAMTS13 may induce platelet-VWF thrombosis in the kidney without appearance of ULVWF in plasma (29). Correcting this imbalance may help prevent or treat acute renal failure in sepsis.

We have recently found that ADAMTS13 may suppress intravascular growth of thrombus (32) and may control thrombosis and inflammation in the microcirculation in brain ischemia, brain reperfusion injury, and myocardial infarction (33–35). These suggested that administration of recombinant ADAMTS13 may correct the imbalance between ULVWF secretion and ADAMTS13 level and may help treat patients with severe imbalance who are at risk for multiple organ dysfunction. In children with thrombocytopenia, multiple organ dysfunction was resolved by restoring ADAMTS13 activity by plasma exchange (17). The VWF-pp/ADAMTS13 ratio may help identify patients with severe sepsis or septic shock at high risk for organ dysfunction because of imbalance between ULVWF secretion and ADAMTS13. Furthermore, this ratio may help identify patients susceptible for organ failure due to endothelial dysfunction in other diseases. Although the present prospective study was limited to few patients who had sepsis and thrombocytopenia, some trends were observed, and larger, controlled, prospective studies are necessary to evaluate and validate these findings.

CONCLUSION

The present study showed simultaneous changes in the levels of ADAMTS13, VWF-pp, VWF, and VWF-pp/ADAMTS13 ratio in patients during the first week of severe sepsis or septic shock. The ratio of VWF-pp/ADAMTS13 was associated with disease severity more than isolated VWF-pp or ADAMTS13 levels. Further studies may show whether organ failure may be prevented by identifying patients with abnormal VWF-pp/ADAMTS13 ratio and restoring the balance between VWF-pp and ADAMTS13 with plasma exchange or recombinant ADAMTS13.

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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).

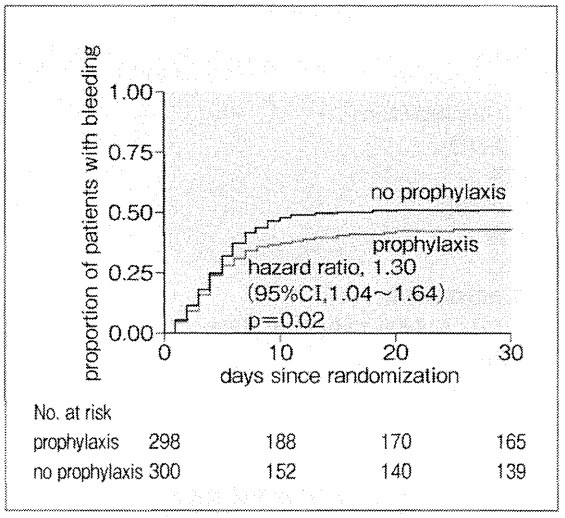

図1 造血器腫瘍患者における予防的血小板輸血の有無における出血頻度

(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 m*l*/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/d*l* 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 m*l*/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 m*l*/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 m*l*/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 m*l*/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 m*l* 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.

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—【報　　告】— Report —

2012年日本における輸血管理及び実施体制と血液製剤使用実態調査報告

牧野　茂義[1)]　田中　朝志[2)]　紀野　修一[3)]　北澤　淳一[4)]　津野　寛和[5)]
佐川　公矯[6)]　髙橋　孝喜[7)]　半田　　誠[8)]

2012年調査は，日赤より輸血用血液製剤が供給された11,348施設に対し実施され4,812施設(42.4%)から回答が得られた．輸血管理体制の整備は，300床以上の医療施設では，輸血責任医師の任命以外は90%以上の実施率であり，ほぼ達成されていたが，小規模医療施設では50～70%の整備率であり，過去5年間はほとんど変化がなかった．特に輸血責任医師の任命は50.3%と低かった．2011年に輸血管理料の施設条件が変更になったため，今回調査では取得施設が急増した．輸血検査では，小規模施設において院外の検査機関に委託する施設が30%前後存在していた．2012年は病床当たりの各血液製剤使用量は昨年と比べて微増程度であったが，2008年調査と比較すると赤血球製剤15.6%，血小板製剤21.5%，新鮮凍結血漿(FFP)30.1%の増加率であった．また，都道府県別の血液使用量は，依然として2～5倍の差を認めた(赤血球製剤2.1倍，血小板製剤4.1倍，FFP 4.4倍，アルブミン製剤4.1倍，免疫グロブリン製剤5.1倍)．今後は，輸血実施施設の90%を占める小規模施設における輸血管理体制の整備を進め，血液製剤の使用量の地域差を少なくすることが重要な課題である．

キーワード：輸血アンケート調査，輸血管理体制，適正輸血

はじめに

「安全な血液製剤の安定供給の確保等に関する法律」(血液法)の基本方針に掲げている適正使用の推進の観点から，「血液製剤の使用指針」及び「輸血療法の実施に関する指針」の徹底が通知されているが，未だ十分周知しているとは言えない．各医療施設における輸血管理及び実施体制の整備と血液製剤の使用状況を正確に把握することを目的に，国の委託事業として血液製剤使用実態調査を実施している[1)~3)]．2012年の調査では，輸血管理体制の整備状況や血液製剤の使用状況を年次別，施設規模別，都道府県別に解析し，輸血検査および実施体制を施設規模別に検討した．尚，本報告内容は2013年11月に開催された平成25年度第1回薬事・食品衛生審議会薬事分科会血液事業部会適正使用調査会で発表したもの[4)]をまとめたものである．

対象および方法

2012年調査は，日本赤十字血液センターより輸血用血液製剤が供給された11,348施設を対象に輸血業務および血液製剤年間使用量調査を依頼した．回答集計および解析を効率的に実施するために，ホームページ上で回答すると電子メールとして自動的に返送され，回収・集計が行われる方式を採用した．病床数別には小規模施設(300床未満)，中規模施設(300～499床)，大規模施設(500床以上)の3群に分けて解析した．本輸血アンケート調査は日本輸血・細胞治療学会(本学会)が中心となって2004年に開始し，当初は300床以上で年間血液使用量が3,000単位以上の全医療施設を含む1,341～1,355施設を対象としていた(表1)[5)~8)]．2005年調査は国が単独で20病床以上の全医療施設を対象に別に実施した．2008年以降は，国の委託事業として本学会が，日本臨床衛生検査技師会および日本赤十字社

1) 虎の門病院輸血部
2) 東京医科大学八王子医療センター臨床検査医学科・輸血部
3) 旭川医科大学病院臨床検査・輸血部
4) 黒石病院小児科・輸血療法管理室
5) 東京大学医学部附属病院輸血部
6) 佐賀県赤十字血液センター
7) 日本赤十字社血液事業本部
8) 慶應義塾大学輸血・細胞療法センター
〔受付日：2013年11月26日，受理日：2013年11月29日〕

表1　過去の輸血アンケート調査実施状況

調査実施年度		2004年	2005年	2005年	2006年	2007年	2008年	2009年	2010年	2011年	2012年
小規模施設	0～19床	0	1	0	2	3	56	100	1,179	1,126	1,349
	20～299床	322	302	3,978	317	301	2,421	1,662	2,427	2,467	2,680
中規模施設	300～499床	280	301	400	306	299	448	341	462	460	497
大規模施設	500床以上	222	241	245	238	241	283	229	284	269	286
調査依頼施設数		1,355	1,355	7,952	1,355	1,342	7,857	7,762	11,435	10,428	11,348
回答施設数		829 (未記入5)	857 (未記入12)	5,452 (記入不備829)	863	844	3,208	2,332	4,352	4,322	4,812
回答率		61.2%	63.3%	68.6%	64.4%	62.9%	40.8%	30.0%	38.1%	41.4%	42.4%
調査実施主体	国（厚労省）			○			国の委託事業として，日本輸血・細胞治療学会が，日本臨床衛生検査技師会および日本赤十字社の協力を得て実施				
	日本輸血・細胞治療学会	○	○		○	○					

の協力を得て実施している[1)~3)]．そのために輸血管理体制の整備状況の年次別推移は，20病床以上施設において，2005年，2008年～2012年において解析し，年次別1病床当たりの血液使用状況は，2008年以降を施設規模別に検討した．

結　果

1. 輸血実施施設の基本項目

2012年調査の回答は4,812施設(42.4%)から得られ，過去5年間で最も高い回答率であった（表1）．200床以上施設の回答率は60%以上であるが，100床未満施設は50%以下であった．回答率の高い県は秋田県(66.0%)，新潟県（61.5%）であり，低い県は鹿児島県(27.1%)，徳島県（27.7%）であった（表2）．小規模施設が4,029施設で全体の83.7%を占めており，病床を持たない施設が512施設（10.6%）含まれていた．実際，2011年に日赤から血液製剤が供給された全施設の中で，病床数が確認できている10,597施設の内訳を図1Aに示す．本邦における輸血実施施設のうち9,523施設(89.9%)は，300床未満施設であった．一方，小規模施設の血液使用量は，赤血球製剤26.2%，血小板製剤12.3%，新鮮凍結血漿(FFP)14.1%であり，多くは300床以上の医療施設で使用されていた(図1B-a，b，c)．本調査に報告された全血液製剤の捕捉率は，日赤から供給された総血液製剤の74.3%であった(図1B-d)．病院の種類としては，医療法人関連病院が1,881施設(39.1%)と最も多く，次に診療所1,207施設（25.1%）が続き，大学病院や国立病院機構等は250施設(5.2%)であった．DPC取得施設は1,090施設（26.2%）であり，病床数別では小規模施設が474施設(14.0%)，中規模施設が359施設（75.4%），大規模施設が257施設（90.2%）であった．輸血管理料取得状況は，輸血管理料Ⅰ取得428施設（10.5%），Ⅱ取得1,049施設（25.7%）であり，2012年の保険改定で輸血管理料取得条件の変更により急速に増加した．

2. 輸血管理体制の整備状況

輸血管理体制の整備状況を2005年，2008年～2012年の調査結果を用いて比較し図2A(20床以上施設)に示す．一元管理がなされている施設は2005年調査では47.2%であったが，2012年には73.6%まで改善し，300床以上施設では92.6%であった．輸血責任医師や輸血担当検査技師の任命も300床以上施設では90%以上の施設で実現している(図2B)．輸血療法委員会の設立は全体では61.4%で達成されており，300床以上施設では95%以上の施設で設立されているが，小規模施設では53.4%であり2008年以降は，あまり進んでいない．

都道府県別に輸血管理体制の整備状況を5項目の整備率の和（輸血管理体制総和）でみた場合（表2），輸血管理体制が最も整備されているのは山形県(407.1%)であり，整備が進んでいない宮崎県（211.7%）と比較すると2倍近い差が認められた．都道府県別に輸血管理体制総和と輸血管理料取得率を表示した場合，その平均値は2011年と比較して右上方に移動しており，輸血管理体制の整備が進み，輸血管理料取得施設が増加していた（図3）．

3. 輸血検査の実施状況

ABO式血液型検査は300床以上施設では，院内の輸血もしくは検査部門の検査技師によって，95%以上の施設で24時間実施されていたが，小規模施設では50～60%の実施率であり，3分の1以上の施設では院外の検査機関に委託していた（図4A）．検査方法は，小規模施設では試験管法が最も多く（69.5%），大規模施設ではカラム凝集法が多かった（84.0%）．検査内容は，オモテ検査は殆どの施設で実施されていたが，ウラ試験は小規模施設では12.9%の施設で未実施であった．交差適合試験の実施状況も血液型検査とほぼ同様の結果であった（図4B）．

輸血前感染症検査として原則的に全て実施している