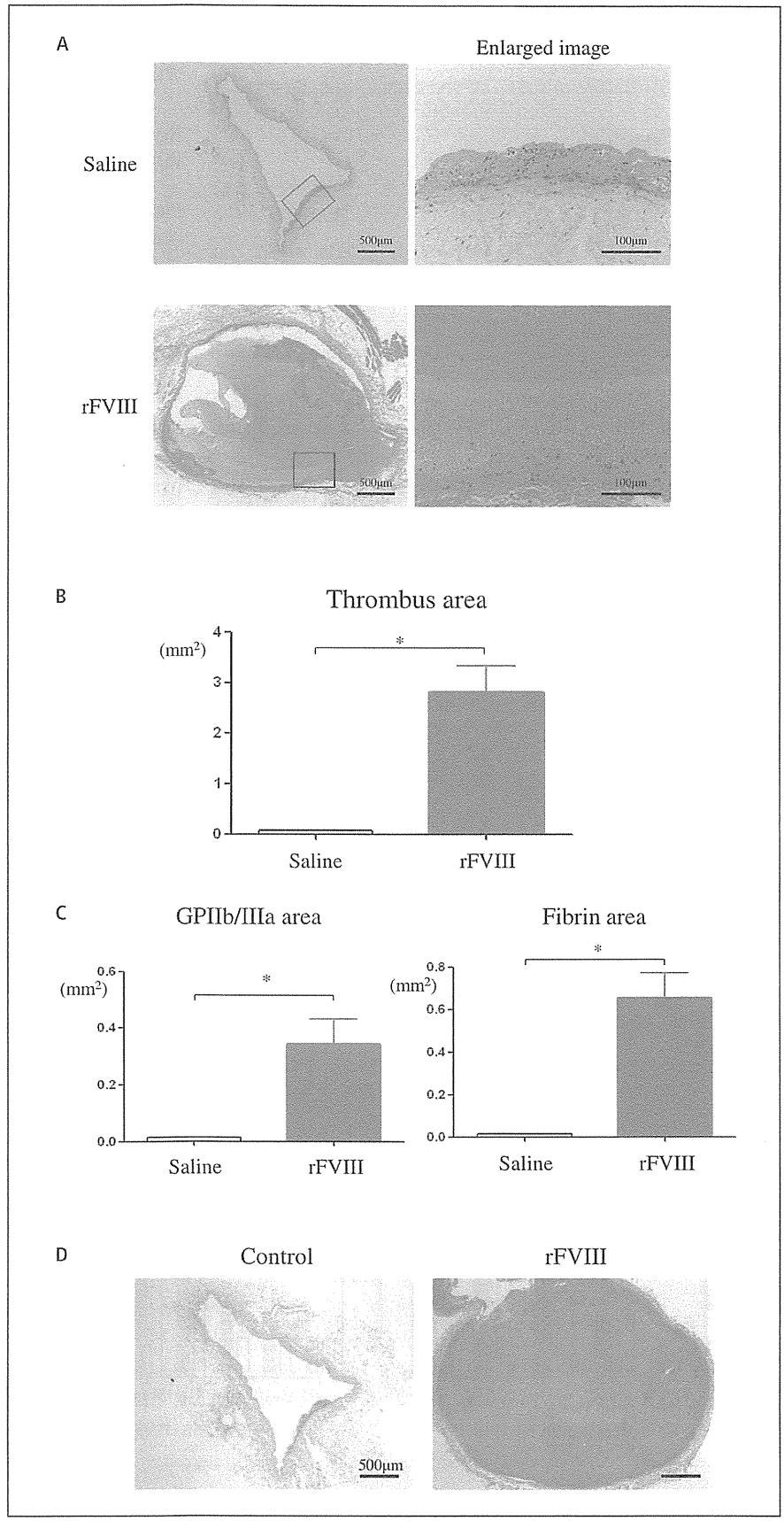

Figure 4: Infused rFVIII enhances thrombus formation in rabbit jugular vein. A) Representative light microphotographs of venous thrombi 1 h after endothelial denudation with or without rFVIII infusion. B) Area with thrombi formed in rabbit jugular veins 1 h after endothelial denudation. Recombinant human FVIII was infused just before endothelial denudation (*$p < 0.0001$, $n = 16$ sections each). C) GPIIb/IIIa and fibrin immunopositive areas in thrombi of jugular veins. Thrombi were immunochemically stained using anti-GPIIb/IIIa and anti-fibrin antibodies (*$p < 0.0001$, $n = 16$ sections each). D) Recombinant human FVIII in venous thrombi localised by staining with anti-human FVIII antibody.

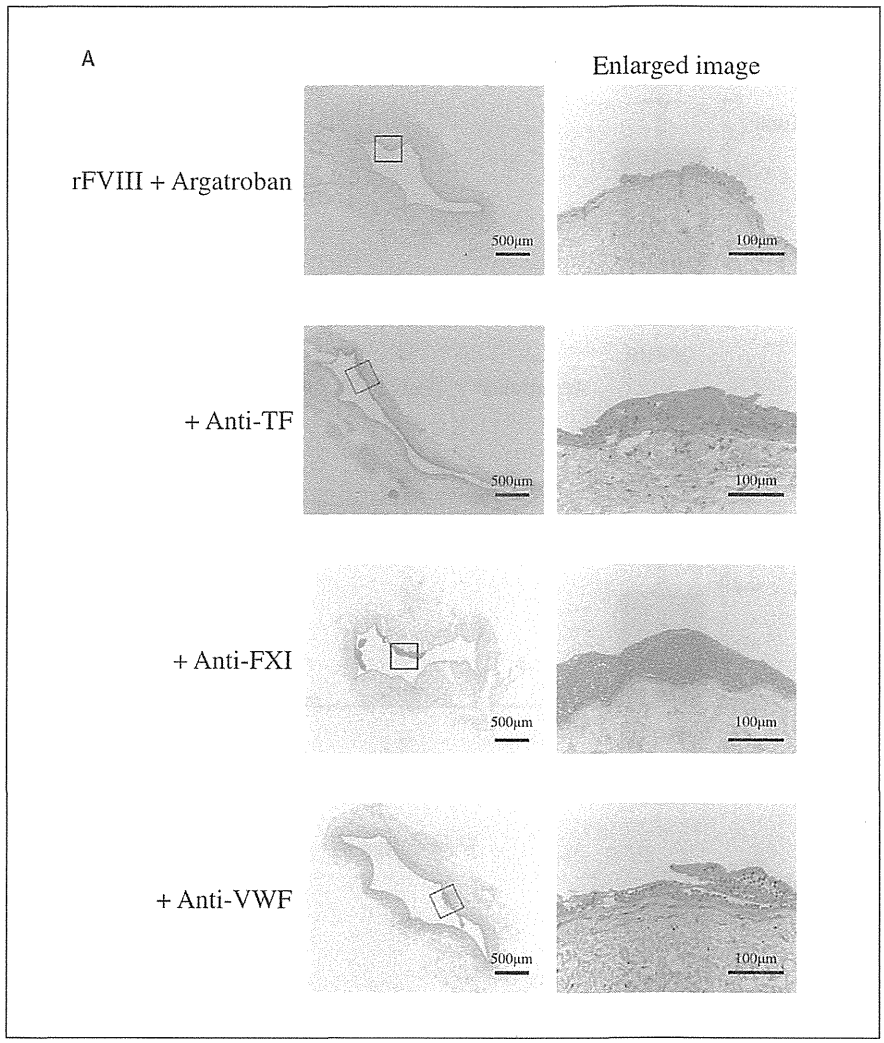

Figure 5: Thrombin, TF, FXI, and VWF are required for thrombus formation enhanced by rFVIII in rabbit jugular veins. A) Representative light microphotographs of venous thrombi 1 h after endothelial denudation that proceeded immediately after infusions of rFVIII and inhibitor of thrombin, TF, FXI and VWF.

Recombinant human FVIII did not affect platelet aggregation

To determine whether rFVIII directly affects platelet function, we assessed the effect of rFVIII on rabbit platelet aggregation initiated by collagen, ADP, botrocetin or thrombin. Recombinant FVIII did not affect platelet aggregation induced by these agents (▶Figure 3).

Recombinant human FVIII did not affect vascular wall thrombogenicity

To determine whether rFVIII directly affects venous wall thrombogenicity, we measured TF activities in rabbit jugular veins 1 h after rFVIII infusion. Rabbit plasma clotting time initiated by the jugular vein homogenate did not differ regardless of rFVIII infusion (104 ± 32 or 108 ± 25 seconds, respectively, p = 0.82, n = 6 each). Levels of TF antigen were 156 ± 20 pg/ml in the venous wall (n = 5), but undetectable in rabbit plasma.

Recombinant human FVIII increased thrombus size in rabbit jugular veins 1 h after endothelial denudation

To determine whether high plasma levels of FVIII promote venous thrombus formation, we histologically assessed rabbit jugular veins after rFVIII (100 IU/kg) infusion. Endothelial denudation caused by balloon insertion induced the formation of small mural thrombus, whereas balloon insertion together with rFVIII (100 IU/kg) infusion enhanced venous thrombus formation (▶Figure 4A) and 11 of 24 jugular veins (46%) became occluded within 1 h. The mean thrombus areas was about 200-fold larger in the rFVIII, than in the control group (▶Figure 4B). The rFVIII infusion alone did not induce either endothelial denudation or thrombus formation.

All venous thrombi were immunopositive for both GPIIb/IIIa and fibrin, and the rFVIII infusion increased both GPIIb/IIIa and fibrin immunopositive areas in thrombi (▶Figure 4C). The ratio of GPIIb/IIIa and fibrin immunopositive to thrombus areas did not differ between the rFVIII-infused and control groups. Throm-

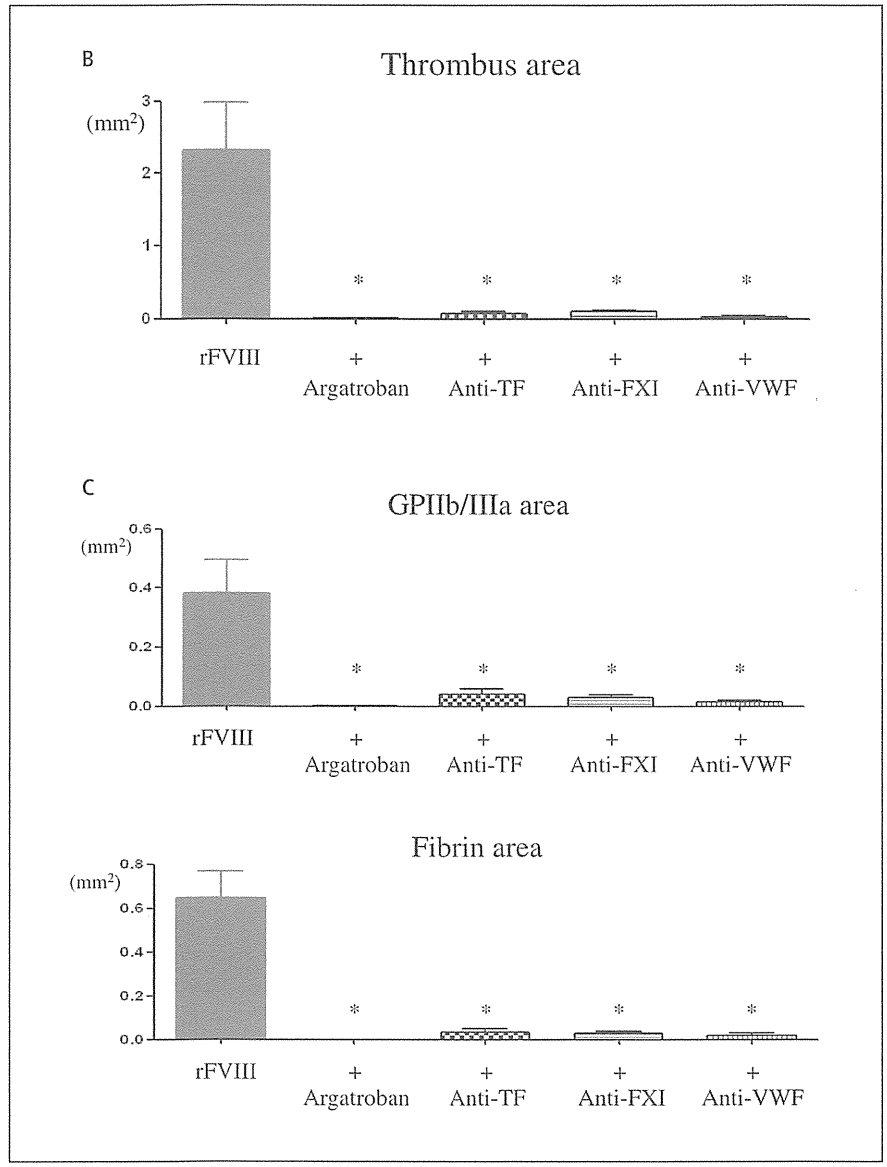

Figure 5 continued: B) Area with thrombi formed in rabbit jugular veins 1 h after endothelial denudation (*p < 0.0001 vs rFVIII group, n = 16 sections each). C) GPIIb/IIIa and fibrin immunopositive areas in thrombi of jugular veins (*p < 0.0001 vs rFVIII group, n = 16 sections each).

bi were immunopositive for human FVIII in the rFVIII-infused, but not in the control group (▶Figure 4D).

Thrombin, TF, FXI, and VWF are required for venous thrombus formation enhanced by rFVIII

We infused rabbit jugular veins with the thrombin inhibitor argatroban, or anti-TF, anti-FXI, or anti-VWF antibodies immediately before endothelial denudation to evaluate the contribution of thrombin, TF, FXI, and VWF to venous thrombus formation enhanced by rFVIII. All of these agents suppressed venous thrombus formation within one hour of endothelial denudation (▶Figure 5A-C).

Thrombin, FXI and VWF are required for FVIII-driven venous thrombus growth but not TF

We assessed the contribution of these thrombotic factors during thrombus propagation in rabbit jugular veins using ICG fluorescence imaging. ▶Figure 6B shows representative images and corresponding fluorescence intensity 15 min after endothelial denudation in the control and rFVIII-infusion groups. Fluorescence emission elicited by ICG in each ROI immediately disappeared in the control group after the ICG infusion (▶Figure 6B and C; Suppl. Movie 1, available online at www.thrombosis-online.com), but persisted in jugular veins due to ICG incorporation by thrombi in the rFVIII group. Thus the fluorescence intensity was significantly higher in the rFVIII, than in the control group (▶Figure 6B and C, Suppl. Movie 2, available online at www.thrombosis-online.

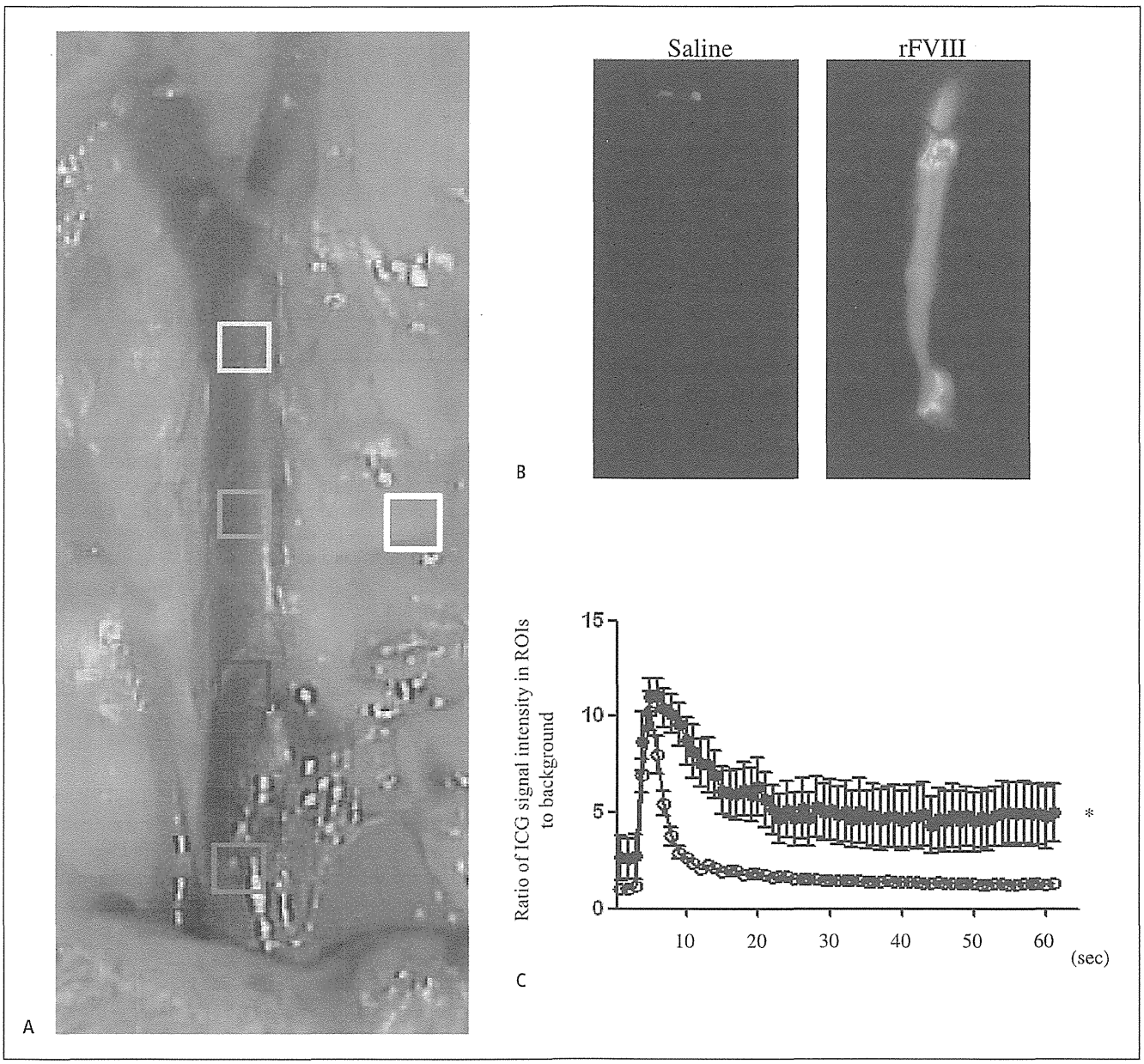

Figure 6: Fluorescence intensity of ICG at 15 min after endothelial denudation. A) Four ROIs (yellow, green, blue, and pink frames) were set at regular intervals on rabbit jugular vein before rFVIII infusion. One adjacent ROI (white frame) was set beside ROIs as background. B) Fluorescent images at 15 min after endothelial denudation and at 15 sec after ICG infusion with saline (left) or 100 IU/kg of rFVIII (right). C) Ratio of fluorescence intensity in ROIs to background after infusing ICG into saline (open circles) and rFVIII (closed circles) groups (*$p < 0.05$ vs control, $n = 4$ each).

com). ▶ Figure 7 shows the average fluorescence intensity in ROIs before and after endothelial denudation in each group. Fluorescence emission gradually became more intense in the rFVIII-infused group, but did not change in the control group. We investigated the relationship between fluorescence intensity and thrombus formation by comparing fluorescence intensity in ROIs with thrombus size in corresponding histological sections. The ratio of ICG fluorescence intensity to the background positively correlated with thrombus area ($r = 0.84$, $p < 0.0001$, $n = 4$).

We administered argatroban, anti-TF, anti-FXI or anti-VWF antibodies when fluorescence intensity exceeded three-fold the background level to evaluate the role of thrombin, TF, FXI and VWF during venous thrombus propagation in the presence of high FVIII levels. At this point thrombi occupied about one sixth of the area of the vessel lumen. The amount of time taken to infuse argatroban, anti-TF, anti-FXI and anti-VWF antibodies did not significantly differ (11 ± 6, 15 ± 6, 13 ± 3, and 14 ± 5 min, respectively). An infusion of argatroban or anti-FXI antibody initially

prevented the increase in the fluorescence intensity, and then gradually reduced the intensity (Suppl. Movies 3 and 4, available online at www.thrombosis-online.com). Anti-VWF antibody also suppressed the increase in intensity, but to a lesser extent than argatroban or anti-FXI antibody (▶Figure 8A), whereas anti-TF antibody did not affect fluorescence intensity (▶Figure 8A, Suppl. Movies 5 and 6, available online at www.thrombosis-online.com). These findings indicate that argatroban and antibodies for FXI or VWF significantly suppressed thrombus propagation, whereas anti-TF antibody did not. Histological and immunohistochemical studies also showed that argatroban and antibodies for FXI or VWF significantly reduced areas of thrombus, platelet (GPIIb/IIIa) and fibrin in thrombus 1 h after endothelial denudation. Anti-TF antibody slightly suppressed thrombus and fibrin areas, but the difference did not reach significance (▶Figure 8B and C).

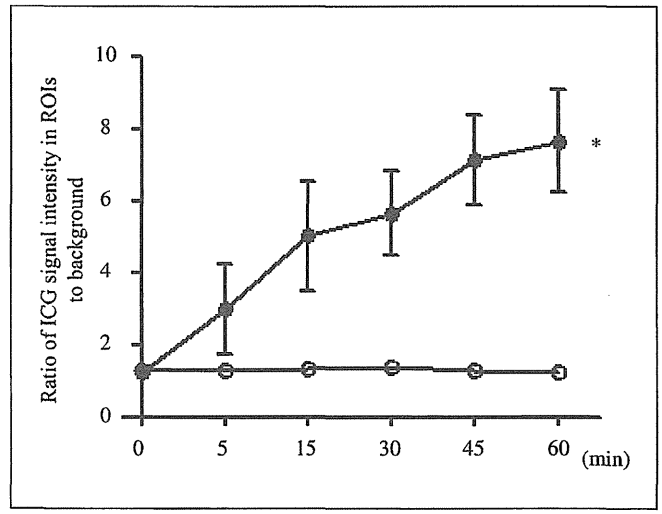

Figure 7: Ratio of ICG to background fluorescence intensity before and after endothelial denudation. Saline (open circles) or 100 IU/kg of rFVIII (closed circles) was infused immediately before endothelial denudation. This graph shows average fluorescence intensity before, and 5, 15, 30, 45 and 60 min after endothelial denudation (*$p < 0.01$ vs control, $n = 4$ each).

Discussion

The present findings showed that elevated levels of FVIII enhance thrombin generation in plasma and promote thrombus formation and propagation in the injured jugular veins of rabbits. Thrombin, FXI, and VWF also significantly contributed to thrombus propagation.

Studies have historically focused on a deficiency of FVIII in patients with haemophilia associated with a significant bleeding diathesis. However, increasing evidence suggests that high plasma FVIII levels might constitute a clinically important risk factor for thrombosis. Several cohort and case-control studies have confirmed a high prevalence of elevated FVIII levels and coagulant activities in patients with DVT or PE and that the increased risk of VTE is dose-dependent upon plasma FVIII levels (3-5, 24, 25). Although levels of plasma FVIII activity widely vary, they are significantly higher in patients VTE than controls (200.1 ± 75.9% vs 151.9 ± 57.7%) (9). The present study found that an infusion of rFVIII elevated rabbit plasma FVIII activity to 200%. This increase is within the range of controls and patients with DVT and corresponds to a five-fold increase in the risk of DVT (3). The aPTT of the rabbit decreased by 60 min, and normalised 120 min after rFVIII infusion. Although the reason is obscure, it could be due to the sensitivity of the aPTT assay and interspecies differences with respect to the half-life of rFVIII. Only normal rabbits were included in this study, which might have made shortening the aPTT difficult. The half-life of rFVIII varies according to species, being 4.1, 5.5 and 15.8 h in mice, rats, and humans, respectively (26).

High plasma levels of FVIII promote venous thrombosis and FVIII inhibition reduces venous thrombosis in mice model with $FeCl_3$-induced thrombosis (11, 12). However, the $FeCl_3$-injury model is to a large extent dependent on blood platelet activation and venous wall injury. Machlus et al. reported that elevated FVIII did not affect carotid arterial thrombus formation after extensive vascular damage caused by $FeCl_3$ (12). Slow or static blood flow together with endothelial injury comprises a fundamental risk factor for DVT. We therefore used a more accurate model of DVT pathophysiology and found that high FVIII levels enhanced venous thrombus formation and propagation. The results also indicated that endothelial denudation alone is insufficient to generate large thrombi even under slow flow, and that combination with a hypercoagulability state is essential for thrombus formation resulting in overt VTE/PE.

A cohort study demonstrated that persistently high FVIII activity actually increased thrombin generation in patients with DVT (27). The present study found that infused rFVIII increased whole blood coagulation and total plasma thrombin generation, but did not affect the initiation time of thrombin generation. Elevated FVIII activity might contribute mainly to the propagation of thrombin generation. The rFVIII did not affect platelet aggregation by thrombin *in vitro* (▶Figure 3). The direct effect of rFVIII on platelet aggregation is less likely and excess thrombin generation enhanced by rFVIII plays a critical role in venous thrombus formation and propagation in our model.

FVIII is recruited by binding to VWF on surfaces comprising collagen and thrombus (28). We previously reported that FVIII colocalises with VWF in venous thrombi of patients with DVT and that FVIII inhibition reduces thrombus formation under low shear conditions *in vitro* (10). Interrupting VWF-platelet interaction prevents venous thrombus formation in rabbit and mouse models of DVT (29, 30). A shortage of VWF also reduces $FeCl_3$-induced thrombus formation in mesenteric venules, and rFVIII infusion in mice does not restore thrombus stability (31). These lines of evidence suggest that VWF plays an important role in FVIII-driven venous thrombus formation. The present study showed that anti-VWF antibody, which interrupts interactions between VWF and GPIbα, suppressed venous thrombus formation under high FVIII levels (▶Figure 5A-C). As reported (32), a blockade of VWF and GPIbα interaction abolished platelet adhesion on a collagen sur-

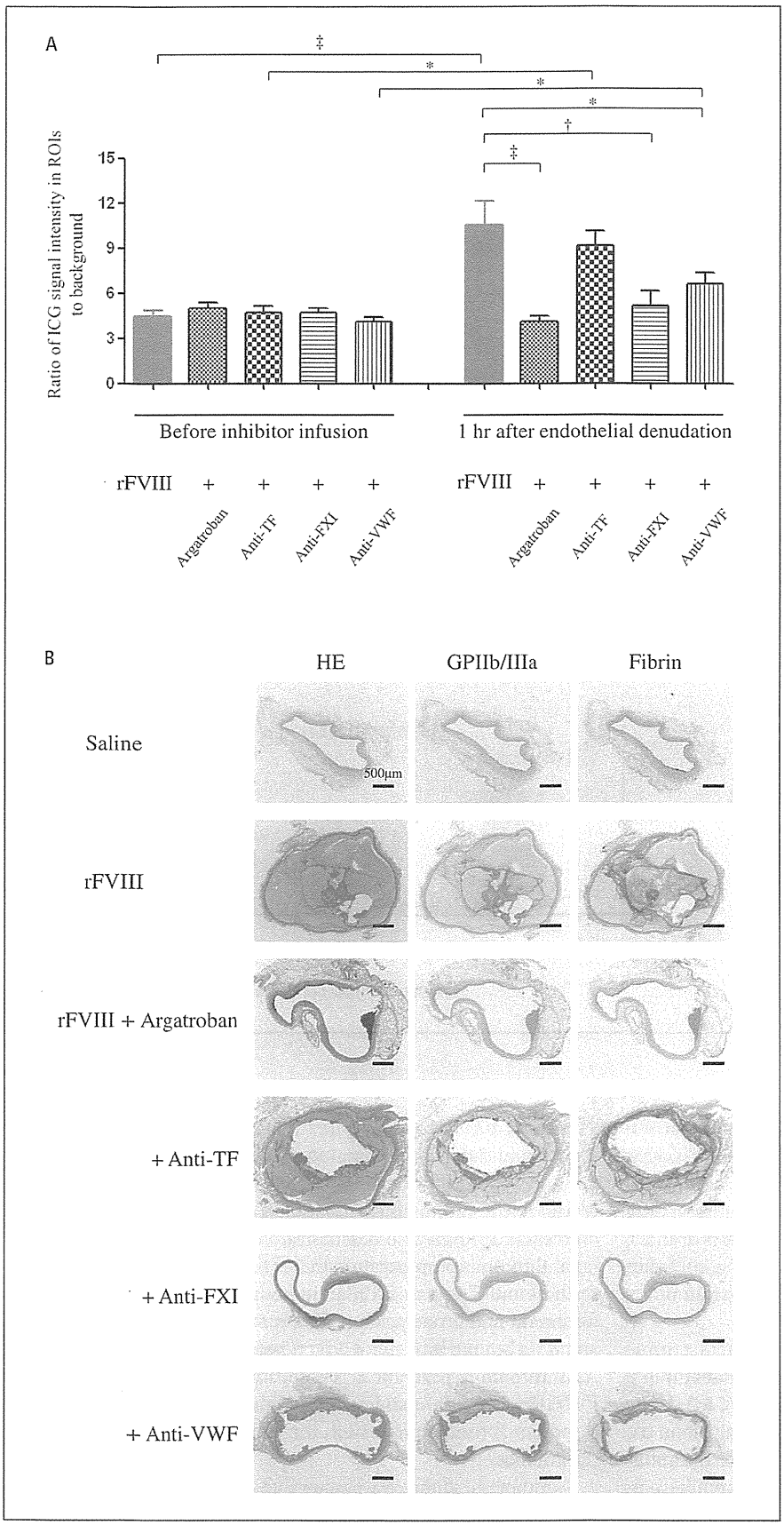

Figure 8: Thrombin, FXI and VWF, but not TF are required for thrombus growth after mural thrombus formation in rabbit jugular vein. A) Venous growth was monitored over time as fluorescence intensity of ICG after endothelial denudation with rFVIII infusion. Argatroban, anti-TF, anti-FXI and anti-VWF antibodies were infused when average fluorescence intensity of ICG exceeded three-fold background. Differences in average fluorescence intensity of ICG before inhibitor administration and 1 h after endothelial denudation are evident (*$p < 0.05$, †$p < 0.01$, ‡$p < 0.0001$, $n = 4$ each). B) Representative light and immunohistochemical microphotographs of venous thrombi. Administration of argatroban, anti-FXI, and anti-VWF antibodies after mural thrombus formation reduced further thrombus formation enhanced by rFVIII infusion.

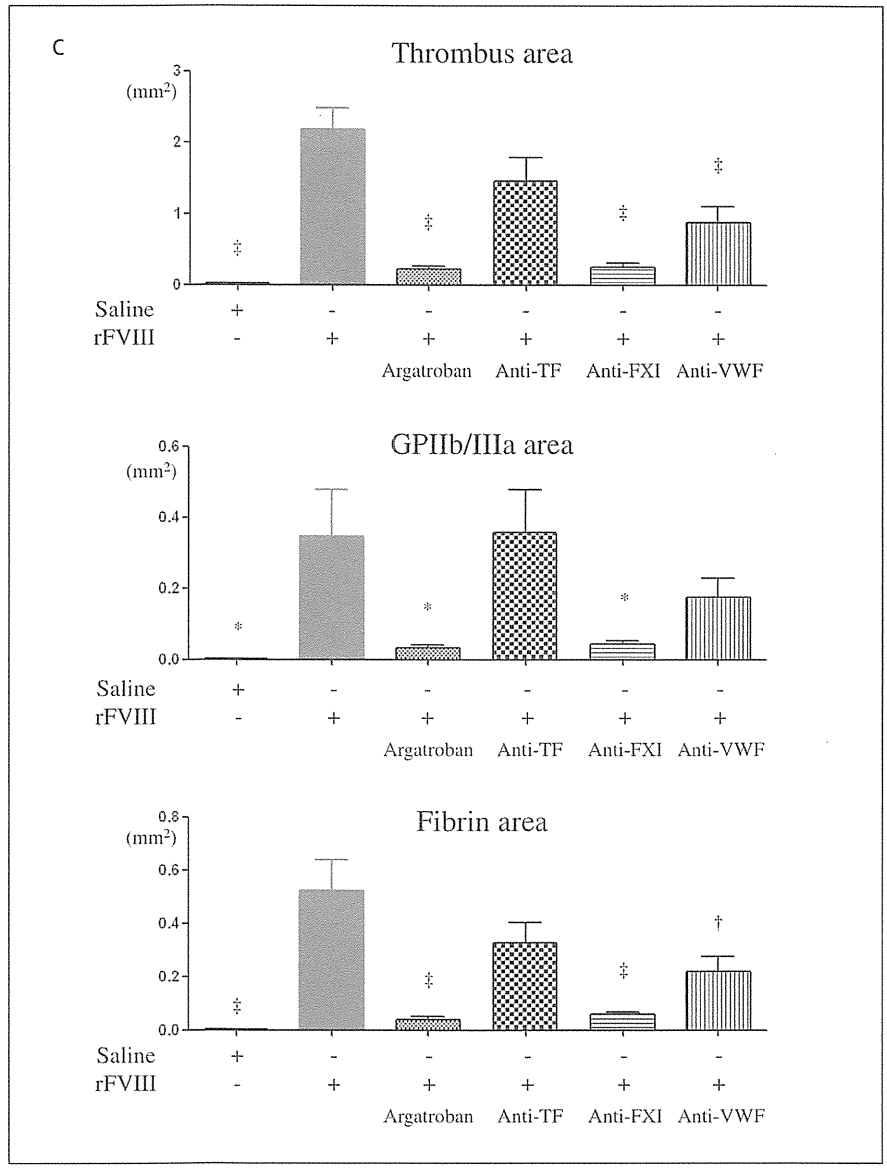

Figure 8 continued: C) Areas of thrombi, and GPIIb/IIIa and fibrin immunopositive areas in thrombi of jugular veins (*$p < 0.05$, †$p < 0.01$, ‡$p < 0.0001$ vs rFVIII group, n = 10 sections each).

face at a high, but not at a low shear rate, indicating that VWF might recruit FVIII on the surface of platelets but not of collagen under conditions of venous flow. This antibody also significantly suppressed venous thrombus propagation, but to a lesser extent than argatroban and anti-FXI antibody (▶Figure 8B and C). These results suggest that VWF-platelet interaction contributes principally to the initiation and somewhat to propagation of venous thrombus under high FVIII levels.

FXI is generally considered to be less important in normal haemostasis, because a bleeding tendency is mild or absent in patients with an inherited or acquired FXI deficiency (33, 34). However, recent studies indicate that FXI is activated during blood coagulation and that even small amounts of FXI induce thrombus growth by generating thrombin and by protecting thrombi from fibrinolysis via thrombin activatable fibrinolysis inhibitor (35, 36). Therefore, FXI apparently plays a significant role in thrombus growth and stability. Animal studies using $FeCl_3$- or vessel clamp-induced venous thrombosis models have shown that FXI plays a crucial role in thrombus propagation and stability (37-39). We and others (20, 40) have also demonstrated that FXI contributes to arterial thrombus propagation rather than to initiation. The present study found that anti-FXIa antibody reduced venous thrombus formation and propagation. FXI is mainly activated by thrombin and FXIa but not by factor XII on negatively charged surfaces (41). As thrombin generation is significantly promoted under high FVIII levels, FXIa could largely contribute to the initiation of thrombus formation as well as thrombus propagation in venous thrombosis.

Although studies have shown that TF contributes to venous thrombus formation and propagation (42, 43), the source of TF in

What is known about this topic?

- Elevated plasma FVIII levels are associated with an increased risk of deep venous thrombosis.
- However, whether elevated FVIII levels promote venous thrombus formation and/or propagation, and its association with other coagulation factors *in vivo* remain unclear.

What does this paper add?

- Elevated plasma levels of FVIII enhance venous thrombus formation and propagation.
- Excess thrombin generation by FXI and VWF-mediated FVIII recruitment might contribute to FVIII-driven venous thrombus growth.

venous thrombosis remains obscure. Mice with a severe TF deficiency have impaired thrombus formation after inferior vena cava ligation (44). Transplanting wild-type mice with low-TF bone marrow does not suppress venous thrombus formation and transplanting wild-type marrow into such mice does not accelerate thrombosis (44). This indicates that vascular wall TF rather than circulating TF is critical for venous thrombus formation. On the other hand, Von Bruhl et al. reported that TF derived from myeloid leukocytes contributes to venous thrombosis initiated by restricting blood flow in the inferior vena cava (45). In addition, TF derived from haematopoietic cells or neutrophils was responsible for thrombus formation and propagation in a laser-induced arteriolar injury model (46, 47). We found here that anti-TF antibody reduced the formation, but not the propagation of thrombus even in the presence of high FVIII levels. Plasma TF protein was undetectable in the rabbits and TF inhibition in blood did not affect whole blood coagulation (data not shown). Our results suggest that venous thrombus formation in this model mainly depends on venous wall TF rather than blood-derived TF. These controversial results could be due to differences among triggers of venous thrombus formation (endothelial denudation, vessel ligation, flow restriction or laser-injury), flow condition (absence, presence or restriction), and vascular bed (jugular vein, inferior vena cava, or arteriole).

Venous thrombi have been created in various animal models. Ferric chloride (48) and electrolytic model (49) are reproducible, but such chemical and physical reactions are far removed from the actual pathophysiology of DVT. Vein ligation with or without endothelial denudation (19, 50) allows the assessment of interaction between the venous wall and progression from acute to chronic thrombus, but has a disadvantage for evaluating the efficacy of therapeutic agents. The vein stenosis model (30) can form laminar thrombus in the presence of blood flow and mimics the clinical situation, but it has the disadvantage of variations in thrombus size and stability. Endothelial denudation without flow restriction induces small venous, but not occlusive thrombi. We applied this model to evaluate thrombus propagation under conditions of elevated FVIII levels.

In conclusion, our results suggest that elevated plasma levels of FVIII enhance venous thrombus formation, and that excess thrombin generation by FXI and VWF-mediated FVIII recruitment might contribute to FVIII-driven venous thrombus growth.

Acknowledgements

This study was supported in part by Grants-in-Aid for Scientific Research in Japan (Nos.23390084, 23790410) from the Ministry of Education, Science, Sports and Culture of Japan.

Conflicts of interest

A. Harada, T. Kitazawa, K. Hattori are employed by Chugai Pharmaceutical Co., Ltd. C. Sugita, A. Yamashita, Y. Matsuura, T. Iwakiri, N. Okuyama, S. Matsuda, T. Matsumoto, O. Inoue, M. Shima, and Y. Asada have no conflicts of interest.

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総 説

急性リンパ性白血病のL-asparaginase療法関連凝固異常に対する国内外の支持療法の現状

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Key words : L-asparaginase, Coagulation disorder, FFP, Antithrombin

緒 言

L-asparaginase（L-asp）は生体内のアスパラギンをアスパラギン酸とアンモニアに分解し，アスパラギンを枯渇させることによってアスパラギン合成酵素を持たない腫瘍細胞の蛋白合成を阻害する薬剤であり，急性リンパ性白血病（ALL）治療のkey drugである。L-aspの重要な有害事象のひとつに凝固異常があり，antithrombin（AT）やfibrinogen（FG）の減少はしばしば観察される。これら凝固異常による血栓症や出血を予防するために，本邦では新鮮凍結血漿（fresh frozen plasma: FFP）やAT濃縮製剤がしばしば投与されてきたが，欧米では予防的FFP補充はあまり行われておらず，L-asp治療中の小児の検討ではFFP投与は凝固異常の改善に有益でないとの報告もある[1]。また，血漿成分のほぼ全ての成分を含むFFPにはFGだけではなく，アスパラギンも含まれるため，アスパラギン枯渇により効果を発揮するL-asp治療中のFFP輸注はL-aspの効果を減弱させる可能性がある[2]との指摘もある。

目 的

本邦におけるL-asp治療中の凝固異常に対する支持療法の実施状況，海外との差異を調査し，検討する。

方 法

日本小児白血病リンパ腫研究グループ（Japanese Pediatric Leukemia/ Lymphoma Study Group: JPLSG）および日本成人白血病研究グループ（Japan Adult Leukemia Study Group: JALSG）のホームページに掲載されている参加施設を対象に，実務担当者宛に往復はがきを送付し，L-aspを含むALL治療中の凝固異常に対する以下のアンケートを行った。

Q1-1. 血栓症や出血予防目的にFFP投与を行っているか

Q1-2. 行っている場合FFP投与の目安となるFG値はいくつか

Q2-1. 血栓症予防目的にATの投与を行っているか

Q2-2. 行っている場合AT投与の目安となるAT値はいくつか

Q3. FFPとAT以外に血栓症予防目的で投与する薬剤はあるか

また，同様の質問を海外の11の小児ALL治療施設にもe-mailにて送付し，回答を得た。

結 果

国内アンケート回収率は，小児JPLSG施設が123/180施設（68%），成人のJALSG施設が100/201施設（50%）であった。血栓症や出血の予防目的にFFP投与を行うと回答した施設は小児で57/123施設（46%），成人では86/100施設（86%）であった。FFP投与基準を図1に示す。成人では70/86施設がFG 100 mg/dl未満と回答したが，小児施設では100 mg/dl未満と答えた施設と50 mg/dl未満と答えた施設が各21施設ずつであった。一方，AT予防投与は小児では114/123施設（93%）で行われていたが，成人では63/100施設（63%）と小児比べて少なかった。図2に示すようにATの投与基準は小児と成人でほぼ同様の傾向であり，AT 70%未満が最多であった。

海外11施設（回答率100%）では，FG値を指標にFFP補充を行う施設は1施設（FG：50>）のみ，ATを値のみを指標として予防的補充を行う施設は3施設

受付：2012年7月30日
受理：2012年12月18日
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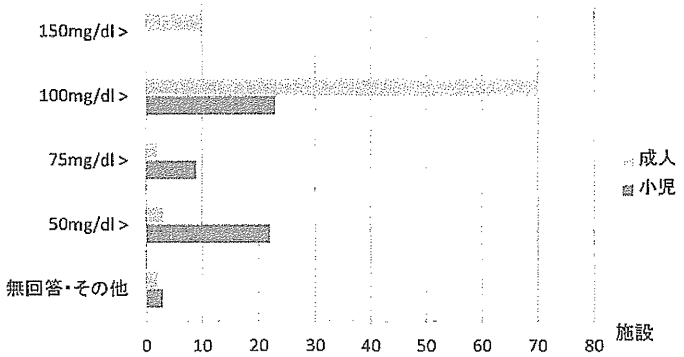

図1 FFP 投与基準
FFP 補充の基準は成人ではほとんどが 100 mg/dl 未満，小児では 100 mg/dl 未満と 50 mg/dl 未満がほぼ同数。

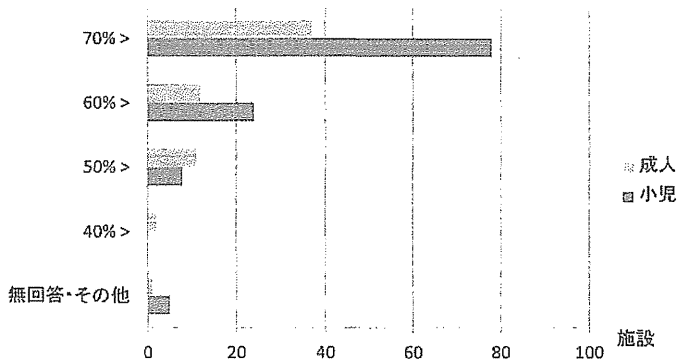

図2 AT 投与基準
AT の補充基準は成人でも小児でも，70%未満が最多で，ほぼ同様の傾向。

(AT：70>，50>，40> 各 1 施設) であった。L-asp 治療中であっても，凝固線溶系因子の定期的測定を行わないと回答した施設も 6 施設 (55%) あった。

また，FFP や AT 製剤以外に血栓症予防を目的に投与する薬剤があるかどうかの設問には，回答した国内 212 施設の 76%がなしと回答，ヘパリンまたは低分子ヘパリン (LMWH) の併用を行っている施設は 18% (39/212) であった。海外では 3 施設が血栓症発症後は LMWH を使用すると回答した。

考 察

本邦では凝固異常による血栓症や出血等の合併症を避けるために，FG や AT の低下に対する補充療法が 80 年代から行われており，本調査でも成人施設の 86%，小児施設の 46%が FFP の，また，成人施設の 64%，小児施設の 93%が AT の予防的投与を行っていた。一方，海外小児施設においては FFP と AT の予防投与実施率は 9%と 27%であった。

「血液製剤の使用指針」の FFP の適正使用の項には「低フィブリノゲン血症 (100 mg/dl 未満) の場合● DIC ● L-アスパラギナーゼ投与後」と記され，FFP は主に出血予防として使用される。しかし，L-asp 治療中の凝固異常に起因する合併症の多くは中心静脈カテーテル関連の血栓症であり，最も危惧される中枢神経系の合併症でも出血は梗塞に続発する梗塞後出血であり，単独の出血の報告はほとんどない。米国で Abbott ら[3] は，FFP またはクリオプレシピテート予防投与を行う IWK Health Center と，同じレジメンで ALL 治療を行うが，いずれの予防投与も行わない B. C. Children's Hospital での後方視的検討を行った結果，予防投与の有無で血栓症発症に有意差がないこと，また，低 FG 血症があっても FFP 補充の有無によらず両群の全 719 例で 1 例も出血がないことを示した。

AT 製剤予防投与の有効性は小児においてはいまだ証明されていないが，成人の CAPELAL study では寛解導入中の AT 製剤の予防投与の血栓症発症率における有効性が，有意差 (予防あり 5% vs なし 13%，$p=0.04$) をもって報告されている。

多くの海外小児施設は，FG や AT 値の測定結果のみに基づく予防的補充療法は行っていない。特に英国では凝固異常の支持療法ガイドラインを定めて，予防的補充療法は実施せず，血栓症発症後の抗血栓治療も urokinase での溶解あるいは LMWH 投与を基本とし，AT 製剤投与は推奨していない。このガイドラインに従った UKALL2003 試験 1,824 例の小児 ALL からは，3.2%が血栓症を起こしたが，一度血栓症を発症した症例もその後は LMWH を併用することで血栓症を再発することなく安全に L-asp 投与継続ができることが報告された[4]。

ステロイドの長期投与が行われる寛解導入の後半には高脂血症もおこりやすく，特に L-asp 投与終了直後には高度の高脂血症も観察される。また，凝固因子に比較して線溶系因子の回復が遅れる傾向があり，血小板数も回復してくる寛解導入終了時期の FFP 投与は血栓症発症

のリスクになりうると考える。FFP 投与直後に血栓症を発症した報告[5] もあり，FG の半減期は FFP に含まれる他の抗凝固，線溶因子より長いため，投与直後は凝固線溶系がアンバランスとなる可能性もある。

結　語

本調査の結果，日本と海外の支持療法の方針には大きな違いがあり，日本では検査値に従った凝固因子補充が行われていることが明らかとなった。

本研究は成育医療研究開発費「小児出血・血栓性疾患診療の向上と均てん化を目的とした治療管理マニュアルの作成と教育研修法の開発」（22 指-3）分担研究と「小児白血病治療に伴う凝固障害の病態・治療法の解明とマニュアル化に関する研究」の研究助成により実施された。

謝辞：アンケートにご協力くださいました各施設の先生方に深謝いたします。

著者の COI（conflicts of interest）開示：本論文発表内容に関連して特に申告なし

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Current national and international status of supportive therapy for the coagulopathy associated with L-asparaginase containing regimen for acute lymphoblastic leukemia

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Key words : L-asparaginase, Coagulation disorder, FFP, Antithrombin

We investigated supportive therapy against coagulopathy associated with L-asparaginase treatment in patients with acute lymphoblastic leukemia who were enrolled in the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG), Japan Adult Leukemia Study Group (JALSG), and foreign institutes. Fresh frozen plasma (FFP) was administered as a supplement in 46% patients in the JPLSG and 86% in the JALSG. The threshold level of FFP infusion was less than 100 mg/dl plasma fibrinogen in 70% of the JALSG and 20% of the JPLSG, while in another 20% of the JPLSG, FFP was administered when the fibrinogen level was less than 50 mg/dl. The preventive use of antithrombin products (AT) was prescribed in 93% of the JPLSG and 63% of the JALSG: The threshold level of AT supplementation was less than 70% of plasma antithrombin activity, which was similar in both groups. Most foreign institutes do not routinely use FFP or AT.

Journal of Perinatology (2013) 33, 239–241

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PERINATAL/NEONATAL CASE PRESENTATION

Neonatal asphyxia and renal failure as the presentation of non-inherited protein C deficiency

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Inherited or acquired protein C (PC) deficiency leads to thromboembolic events. Plasma PC activity in infancy is physiologically lower than in adults. We describe a case of neonatal asphyxia and acute renal failure associated with isolated PC deficiency. A full-term male infant was born to a healthy mother by caesarean section because of fetal distress. The small-for-gestational age infant showed 2 and 7 of Apgar scores at 1 and 5 minutes, respectively. Hypercoagulability required repeated infusions of fresh frozen plasma. Coagulation study revealed PC activity, 6%, protein S activity, 61%, and high D-dimer levels, along with normal factor VII activity and absent vitamin K deficiency. Anticoagulant and activated PC therapy improved coagulopathy and nephropathy. Imaging analyses indicated no visceral infarctions. Renal function and PC activity have been slowly normalized until 6 months of age. He had no *PROC* mutation or PC-deficient parents. Selective PC deficiency may occur as an acquired cause of hypercoagulable crisis in the stressed newborn.

Journal of Perinatology (2013) **33**, 239–241; doi:10.1038/jp.2012.55

Keywords: protein C deficiency; purpura fulminans; activated protein C therapy

Introduction

Protein C (PC) is a vitamin K-dependent serine protease zymogen produced by the liver. The anticoagulant factor is activated by the thrombin (factor IIa: FIIa)-bound thrombomodulin expressed on the endothelium. Activated PC (aPC) inactivates FVa and FVIIIa in the presence of protein S (PS) as the cofactor.[1] Administration of aPC products inhibits clot formation and augments fibrinolysis by blocking plasminogen activator inhibitor-1. aPC also exerts anti-inflammatory and cytoprotective effects on neutrophils, macrophages, dendritic cells and endothelial cells via specific receptors.[2] Inherited PC deficiency is an autosomal recessive thrombophilia.[1] Homozygotes and compound heterozygotes of *PROC* mutation present with purpura fulminans (PF) during early neonatal period.[3] Heterozygous *PROC*-mutated adults are at risk of deep vein thrombosis, pulmonary thromboembolism and disseminated intravascular coagulopathy, being often triggered by infection, vasculopathy and malignancy. Sepsis and liver dysfunction precipitate PC deficiency via the consumption, impaired synthesis and both. Plasma PC activity is physiologically low until 6 months of age. Neonates are vulnerable to vitamin-K-deficient coagulopathy arising from the immaturity, nutrition and enteral microbiota. Nevertheless, there is little information about neonatal thromboembolism arising from non-familial PC deficiency.

We report a case of neonatal asphyxia and renal failure associated with non-inherited PC deficiency. The etiology and management of hypercoagulable crisis in the newborn was discussed.

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Received 10 November 2011; revised 30 January 2012; accepted 3 April 2012

Case

A male newborn was hospitalized because of neonatal asphyxia showing 2 and 7 of Apgar scores at 1 and 5 min, respectively. He was born to a healthy 30-year-old mother, gravida 0 and para 0, at 41 weeks of gestation by urgent caesarean section for non-reassuring fetal status on the labor induction, after uneventful pregnant course. The small-for-gestational age infant weighing 2404 g had normal umbilical cord and placenta. There was no consanguineous marriage or contributory family history. On day 6 after birth, he was transferred to our tertiary neonatal intensive care unit because of anuria and thrombocytopenia.

On admission, the vigorous infant showed 70 min^{-1} of tachypnea, normal pulse rate (136 min^{-1}) and blood pressure (89/67 mm Hg). Body temperature was 37.6 °C. There was no desaturation. He had occipital cephalhematoma and faint purpura (3 × 5 cm) on the right back. Auscultation was unremarkable. No hepatosplenomegaly was found. Complete blood counts and coagulation study 2 days after the transfusion of packed platelets and fresh frozen plasma (FFP) revealed white blood cells

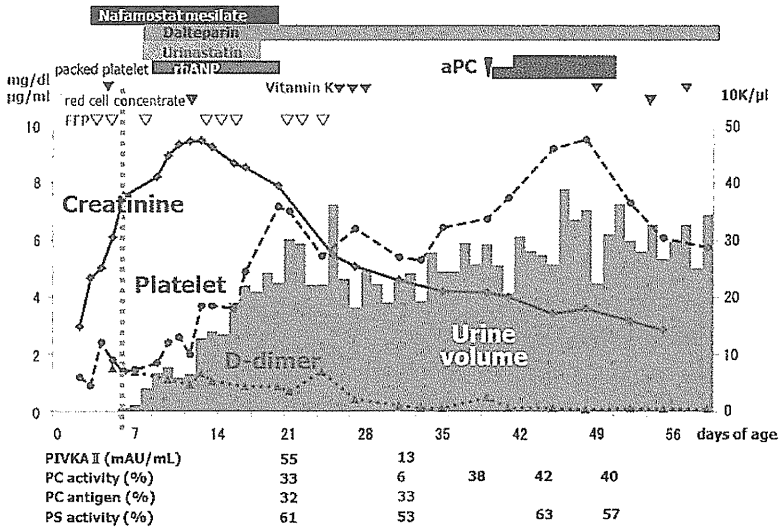

Figure 1 Treatment course of the infant. Intensive therapy was directed to anticoagulant and nephroprotective effects. Repeated infusions of fresh frozen plasma (FFP) increased platelet counts and urine volume. Serum creatinine peaked at 9.47 mg dl^{-1} and slowly decreased. The activated protein C (aPC) therapy further improved platelet counts, D-dimer and creatinine levels. PIVKA-II, protein induced by vitamin K absence or antagonists-II; PS, protein S; rhANP, recombinant human atrial natriuretic peptide.

7.540 × 10^9 l^{-1}, hemoglobin 9.9 g dl^{-1}, platelets 69 × 10^9 l^{-1}, fibrinogen 103 mg dl^{-1}, prothrombin time-INR 1.56, activated partial thromboplastin time 48.1 s, fibrinogen degradation products 15.0 μg ml^{-1} and D-dimer 7.9 μg ml^{-1}. Antithrombin activity was 47%, thrombin–antithrombin complex was 7.3 ng ml^{-1} and plasmin α2–antiplasmin complex was 2.5 μg ml^{-1}. C-reactive protein concentration was 0.13 mg dl^{-1}. Serum levels of creatinine (7.54 mg dl^{-1}), urea nitrogen (59 mg dl^{-1}), uric acid (22.5 mg dl^{-1}), aspartate aminotransferase (113 U l^{-1}), alanine aminotransferase (345 U l^{-1}) and creatinine kinase (904 U l^{-1}) were high. Blood gas analysis indicated metabolic acidosis with −7.1 mmol l^{-1} of actual base excess. Dark red urine indicated hemolysis. Echographies revealed intact brain, heart, liver and kidney.

Intensive therapy was started for renal failure and disseminated intravascular coagulopathy (Figure 1). Dalteparin, nafamostat mesilate, urinastatin, antithrombin products and FFP but not packed platelets were infused for the hypercoagulability of unknown cause. Diuretics, catecholamine, recombinant human atrial natriuretic peptide and packed red cells were administered. Urine volume slowly increased. Serum creatinine levels at the peak of 9.47 mg dl^{-1} on day 13 of age decreased gradually. During repeated FFP infusions, low PC activity (33%, reference range (rr): 67–130) was dissociated with subnormal PS activity (61%, rr: 73–121) on day 21 of age. The steady improvement of coagulopathy and renal function after each FFP substitution was suggestive of inherited thrombophilia. The coagulation profile was reassessed after vitamin K therapy and 10 days after the last FFP infusion. PC activity was 6%, PC antigen level was 33% (rr: 65–135) and PS activity was 53%. Protein induced by vitamin K absence or antagonists-II levels (PIVKA-II; 13 mAU ml^{-1}, rr: ⩽40) and FVII activity (75%, rr: 75–140) were normal. These findings indicated the type 2 PC-deficient heterozygote. Administration of human plasma-derived aPC (Anact C; a bolus infusion of 50 U kg^{-1} and a continuous infusion of 50–100 U kg^{-1} for consecutive 7 days, The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan) led to the drastic increase of platelet counts, undetectable D-dimer and decreasing creatinine levels. Magnetic resonance imaging of the brain and abdomen, scintigraphy of the lung and heart, renogram and funduscopy revealed no infarctions. Direct sequencing of the coding and promoter regions of *PROC* revealed no mutation in the infant.[4] Healthy father and mother had normal PC activity of 135% and 165%, respectively. Thereafter, PC activity of the patient increased to 55% until the age of 3 months, and attained 102% at the age of 10 months. Serum creatinine levels fell to 0.87 mg dl^{-1} until 10 months. The infant shows normal development at the writing of the manuscript.

Discussion

The hypercoagulable neonate presented renal failure, cephalhematoma, purpura and hemoglobinuria. Extremely low PC but not PS activity, the response to aPC therapy and no *PROC* mutation determined the diagnosis of acquired PC deficiency. Despite the low frequency of *PROC* mutation, the thrombotic risk of PC mutant is higher than other congenital thrombophilias.[5,6] Neonatal PF is the hallmark of severe PC deficiency (<0.01 U ml^{-1} or <1% of PC activity). Heterozygous PC-deficient infants are at

risk of PF in the association with additional triggers.[4] Cerebral infarction and/or vitreous bleeding were the other presentations of fetal/neonatal PC deficiency.[7] Kidney is a target organ of thrombosis in neonatal thrombophilias.[8] In this context, selective PC deficiency has a greater impact on the thromboembolic events especially in early infancy than other thrombophilias.

The major concern is the etiology of transient PC deficiency in the newborn. Manco-Johnson *et al*[9] described 11 infants initially seen in the newborn period with undetectable PC activity and/or antigen, which proved on subsequent follow-up, to be acquired. About 5 of the 11 infants manifested thrombotic events, including renal, aortic and cerebral sites. They further evaluated PC, D-dimer and other regulatory proteins in 164 newborn infants. In this report,[10] prevalence of PC $<0.1\,U\,ml^{-1}$ or $<10\%$ of healthy adults ranged from 0 to 37% with the highest rates in twin gestation (either term or preterm) and preterm infants with respiratory distress, and including a rate of 5% in term singleton infants with distress. Although they showed no genetic study, severe to moderately severe PC activity ($<0.1\,U\,ml^{-1}$ or $<10\%$) could occur and often led to thrombosis in the stressed newborn as non-inherited PC deficiency.

At the time of diagnosis, the discrepancy between 6% of PC activity and 33% of PC antigen levels was unexplainable. The lower PC activity than PC antigen levels at first suggested type 2 deficiency, which accounts for 15% of symptomatic deficiency. Genetic PC deficiency arises from a gross deletion in patients with no *PROC* mutation.[11] However, the qualitative and quantitative abnormality of PC molecule associated with *de novo* heterozygous PC deficiency was excluded by 102% of PC activity at the age of 10 months. The blood samples at diagnosis showed undetectable PIVKA-II and normal FVII activity. It may raise the possibility that a certain inhibitor selectively reduced the PC activity but not PS activity, while no such factors have been suggested.[12] Shorter half-life of PC and aPC than PS might contribute to persistently low PC activity. To clarify the cause of neonatal PC deficiency, genome-wide study should be directed toward the genotyping associated with the functional maturation of PC activity.

The other issue is the indication of aPC concentrate. The efficacy of aPC therapy is still controversial in the treatment of sepsis and/or PF in childhood.[3,7] During the disease course, the image analyses disclosed no evidence of thrombosis. However, the early anticoagulant therapy protected irreversible organ damages with impaired circulation. After the start of aPC administration, the increase of platelet counts and disappearance of D-dimer were drastic. Subsequent recovery of renal function might corroborate the additional profibrinolytic and cytoprotective effects of aPC on the ischemic kidney. Although genetic study is indispensable for PC deficiency, the entity of neonatal PC deficiency might emphasize the feasibility of aPC therapy beyond the *PROC* mutation. Thromboprophylaxis should be augmented for PC-deficient patients. Warfarin control is not easy in young children. In this line, regular PC substitution may be limitedly recommended in early infancy. Further study is needed to search the non-inherited causes of neonatal isolated PC deficiency as well as to establish the optimal aPC therapy for PC-deficient infants.

Conflict of interest

The authors declare no conflict of interest.

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International Journal of Cardiology xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Thrombocytosis in asplenia syndrome with congenital heart disease: A previously unrecognized risk factor for thromboembolism

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ARTICLE INFO

Article history:
Received 10 March 2012
Received in revised form 15 May 2012
Accepted 7 June 2012
Available online xxxx

Keywords:
Thrombocytosis
Asplenia
Congenital heart disease

ABSTRACT

Background: Thrombocytosis and thromboembolic complications occur after splenectomy. However, there is no previous report investigating the presence of thrombocytosis and its association with thromboembolic events in patients having asplenia syndrome with congenital heart disease.
Methods: Enrolled were 161 consecutive patients with functionally single ventricle who underwent cardiac catheterization between 1997 and 2010. They were divided into two groups: patients having asplenia (Group A, n = 46) and patients having no asplenia (Group B, n = 115). Aspirin therapy was employed in all patients after surgical interventions except for pulmonary artery banding. We retrospectively reviewed the platelet counts at each seven stage of cardiac catheterization (for pre- and postoperative evaluation of the first palliation, Glenn operation, and Fontan operation, and for late evaluation after Fontan operation), incidence of thromboembolic events, and other possible risk factors for thromboembolism.
Results: The median platelet counts in Group A were consistently higher than those in Group B at any of the seven stages of cardiac catheterizations ($p<0.002$). The incidence of thromboembolic complications was also higher in Group A than that in Group B (28% vs. 10%, $p=0.030$). Univariate and multivariate logistic regression analyses showed that a platelet count of more than 550×10^9/L at the first cardiac catheterization was associated with thromboembolic complications (Odds ratio 3.17; $p=0.046$).
Conclusions: Persistent thrombocytosis is present in patients with asplenia syndrome. It may greatly contribute to the development of thromboembolism during the management of congenital heart disease than expected.

1. Introduction

Post-splenectomy thrombocytosis is reported to be a predisposing factor for thromboembolism. Thromboembolic complications following splenectomy occur in up to 10% of patients. These range from myocardial infarction, portal vein thrombosis, and pulmonary embolism to deep vein thrombosis [1–3]. Such thrombotic events could be observed not only immediately after surgery, but also several months or even years later in the patients in whom thrombocytosis persisted [4].

Congenital asplenia syndrome is a form of heterotaxy that is also known as right atrial isomerism. This syndrome is typically associated with severe heart defects and needs staged cardiac operations from infancy as a functionally single ventricle [5]. Thromboembolic events are often experienced before and after the completion of Fontan operation [6].

There are a few reported cases with secondary thrombocytosis caused by isolated spleen agenesis without cardiac defects, mimicking essential thrombocythemia [7,8]. Thromboembolism is one of the major complications during and after surgical intervention in patients with congenital heart disease. However, there is no previous report investigating the relationship between platelet count and thromboembolic events in patients with asplenia syndrome having congenital heart disease.

The objective of this study is to clarify the clinical significance of thrombocytosis and its impact on thromboembolic events in patients with congenital heart disease and asplenia.

2. Materials and methods

2.1. Patients

A total of 161 consecutive patients with functionally single ventricles who underwent cardiac catheterization at Kyushu University Hospital and Kyushu Koseinenkin

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doi:10.1016/j.ijcard.2012.06.009

Table 1
Patient characteristics.

	Group A (n = 46)	Group B (n = 115)
Anatomy of the congenital heart disease		
SRV	19	13
AVSD	14	6
DORV	7	23
SLV	2	15
CCTGA	2	13
TA	0	19
PAIVS	0	10
HLHS	0	8
MA	0	5
Others	0	3
Age	7 years (6 months–13 years)	7 years (4 months–13 years)
Sex	M/F = 22/24	M/F = 57/58
First palliation		
Blalock–Taussig shunt	38 (83%)	66 (57%)
Pulmonary artery banding	8 (17%)	41 (36%)
Norwood operation	0 (0%)	8 (7%)
Survival	33 (72%)	107 (93%)

SRV: single right ventricle, AVSD: atrioventricular septal defect, DORV: double outflow right ventricle, SLV: single left ventricle, CCTGA: congenitally corrected transposition of the great arteries, TA: tricuspid atresia, PAIVS: pulmonary atresia with intact ventricular septum, HLHS: hypoplastic left heart syndrome, MA: mitral atresia.

Hospital between 1997 and 2010 were enrolled in this study. No patients had any family history of thrombophilia. The patients were divided into two groups: patients having asplenia syndrome (Group A, n = 46) and those having no asplenia syndrome (Group B, n = 115). Patient characteristics of each group are shown in Table 1.

All patients underwent staged surgical procedures. Initial palliation included Blalock–Taussig (BT) shunt, pulmonary artery banding or Norwood operation as needed. The second stage operation was a bidirectional Glenn cavopulmonary connection performed on cardiopulmonary bypass. The third stage operation was Fontan operation using expanded polytetraflorethylene vascular graft as an extracardiac conduit. Fenestration was only performed for two patients with high pulmonary vascular resistance. All patients received cardiac catheterization for pre- and postoperative evaluation of each surgical procedure and for late evaluation after Fontan operation (Fig. 1).

Postoperative and interstage anticoagulation therapy was identically performed as follows: aspirin (3–5 mg/kg/day) after BT shunt, Norwood procedure, and Glenn operation; aspirin and warfarin (targeted prothrombin time-international normalized ratio [PT-INR] 1.5–2.0) for 1 year following Fontan operation; and only aspirin after 1 year following Fontan operation.

The diagnosis of asplenia syndrome was determined based on the presence of Howell–Jolly bodies in the peripheral blood smear and the absence of spleen assessed by computed tomography (n = 31) and/or ultrasonography (n = 46). The diagnosis of thromboembolic complications was determined using objective methods when they were suspected by clinical manifestation or when they were coincidentally found by the regular examinations. BT shunt malfunction was defined as shunt occlusion or stenosis which needs catheter intervention or surgery, assessed by computed tomography or cardiac catheterization. Cerebral infarction was diagnosed by computed tomography or magnetic resonance imaging. Diagnosis of venous thromboembolism was made by ultrasonography, computed tomography, or venography.

3. Methods

We retrospectively reviewed the medical records of all 161 patients to determine (i) the platelet counts at each seven stage of cardiac catheterization; (ii) the incidence of thromboembolic complications; and (iii) other clinical data that may be possible risk factors for thromboembolic complications in each group.

For the purpose of elucidating the association between platelet counts and thromboembolic events, we investigated the sequential changes of platelet counts, the timing of thromboembolic events, and the correlations among platelet counts at each stage of cardiac catheterizations.

We evaluated the effect of clinical parameters (platelet count, age, birth weight, hemoglobin concentration, activated partial thromboplastin time, ejection fraction of the systemic ventricle, atrioventricular valve regurgitation, presence or absence of BT shunt, size of the BT shunt, and presence or absence of pulmonary vein obstruction) as a risk factor of thromboembolic complications with univariate and multivariate logistic regression analyses.

3.1. Statistical analysis

Continuous variables were analyzed using the Mann–Whitney *U* test and categorical variables were analyzed using the chi-square test. The analysis of Pearson's correlation coefficient was used to evaluate correlations between platelet counts at each other stage of catheterization. Dichotomous variables were created out of continuous variables by using clinically important cutoff points. Univariate and

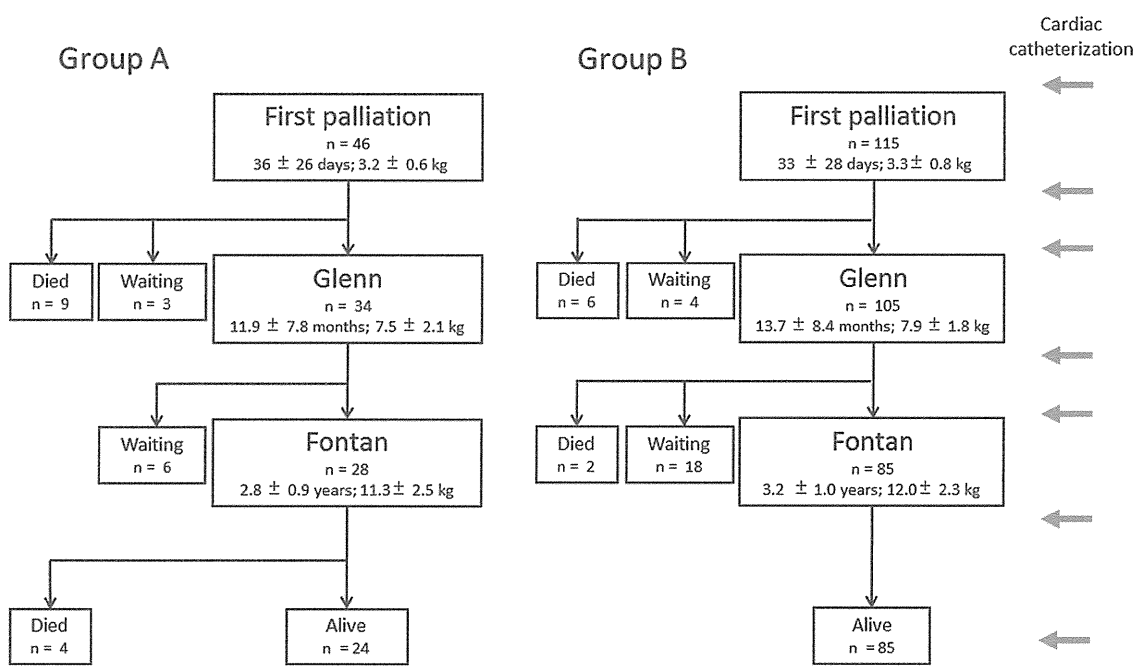

Fig. 1. Number of study patients, mean age and mean weight at each stage.

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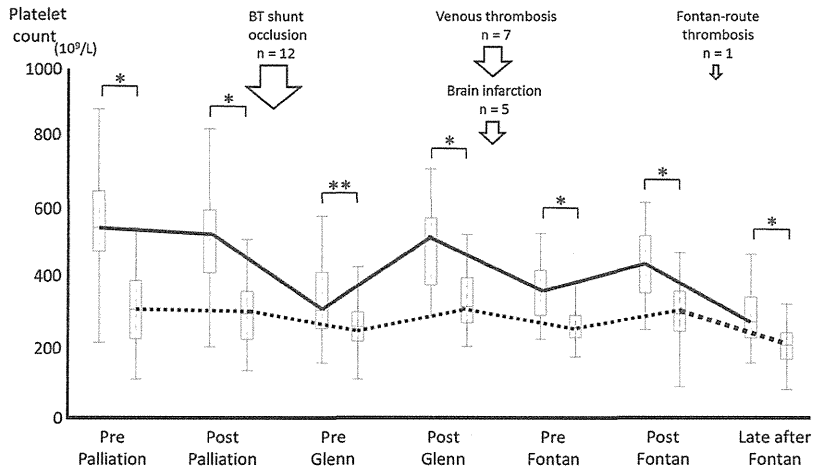

Fig. 2. Sequential changes of platelet counts in Group A (solid line) and Group B (dashed line) at the seven therapeutic stages of cardiac catheterizations and the timing (arrows) of thromboembolic events. The box plots represent medians with 25–75th centile boxes, minimums and maximums still in the 1.5 interquartile range bars, and outliers. The median platelet counts in Group A were significantly higher than those in Group B at any of the seven stages of cardiac catheterizations (* $p<0.0001$, ** $p=0.0002$).

multivariate logistic regression analyses were used to determine the relative contribution of various factors to the risk of thromboembolic events. *P* values of less than 0.05 were considered significant. All statistical operations were performed by using the JMP 8 statistical software package (SAS Institute. Inc. Cary. NC).

4. Results

The time course of the platelet counts and thromboembolic events are shown in Fig. 2. The median platelet counts in Group A were consistently higher than those in Group B at any of the seven stages of cardiac catheterizations (557×10^9/L vs. 333×10^9/L, $p<0.0001$; 543×10^9/L vs. 349×10^9/L, $p<0.0001$; 313×10^9/L vs. 254×10^9/L, $p=0.0002$; 524×10^9/L vs. 304×10^9/L, $p<0.0001$; 310×10^9/L vs. 248×10^9/L, $p<0.0001$; 391×10^9/L vs. 278×10^9/L, $p<0.0001$; and 336×10^9/L vs. 207×10^9/L, $p<0.0001$, respectively). The most frequent thromboembolic event was the BT shunt malfunction in the infant period (large arrow). The other occasional thromboembolic events were venous thrombosis and cerebral infarction after Glenn operation.

The incidence of thromboembolic events was also higher in Group A than in Group B (28% vs. 10%, $p=0.0302$, Fig. 3). Furthermore, patients in Group A were associated with increased incidences of all four types of thromboembolic complications: BT shunt malfunction; cerebral infarction; venous thromboembolism; and Fontan-rout thrombosis.

There were significant correlations between platelet counts of individual patients at each other stage of cardiac catheterization ($p<0.05$, Table 2). On the basis of the correlations, we investigated the possible risk factors for thromboembolic events including platelet count at the first cardiac catheterization as one of the representative values of thrombophilic predisposition.

The univariate logistic regression analysis indicated that patients who had a platelet count of more than 550×10^9/L at the first cardiac catheterization (Odds ratio 3.17; $p=0.023$), or underwent BT shunt (Odds ratio 2.47; $p=0.044$) were at a higher risk of thromboembolic complications. The multivariate logistic regression analysis selected only the platelet count of more than 550×10^9/L as a risk factor of thromboembolic events ($p=0.046$). No other clinical variables including birth weight, BT shunt size, pulmonary vein obstruction, ejection fraction of the systemic ventricle, atrioventricular valve regurgitation, hemoglobin concentration, and activated partial thromboplastin time at the first cardiac catheterization were associated with thromboembolic events (Table 3).

5. Discussion

This is the first report that demonstrated the presence of persistent thrombocytosis and its association with thromboembolic complications in patients with asplenia syndrome. Patients with asplenia syndrome were reported to have poor outcome. Pulmonary vein obstruction, arrhythmias associated with twin atrioventricular nodes, and susceptibility

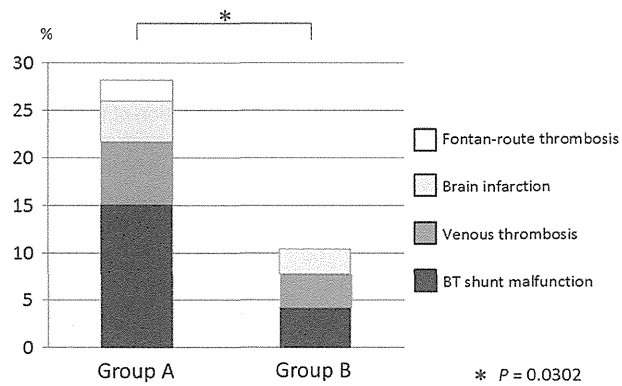

Fig. 3. Incidence of thromboembolic complications in Group A and Group B. The incidence of thromboembolic complications is significantly higher in Group A than in Group B. BT shunt: Blalock–Taussig shunt.

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Table 2
Correlation and coefficient among platelet counts at each stage of cardiac catheterization.

		Pre palliation	Post palliation	Pre Glenn	Post Glenn	Pre Fontan	Post Fontan	Late after Fontan
Pre palliation	R^2		0.2724	0.2179	0.2203	0.1466	0.1266	0.1059
	p Value		<0.0001	<0.0001	<0.0001	0.0002	<0.0001	0.0292
Post palliation	R^2			0.1268	0.1923	0.1489	0.1577	0.2205
	p Value			0.0003	<0.0001	0.0002	0.0002	0.0011
Pre Glenn	R^2				0.2545	0.2018	0.1736	0.3165
	p Value				<0.0001	<0.0001	0.0001	0.0002
Post Glenn	R^2					0.3978	0.2406	0.4329
	p Value					<0.0001	< 0.0001	<0.0001
Pre Fontan	R^2						0.1578	0.318
	p Value						<0.0001	<0.0001
Post Fontan	R^2							0.238
	p Value							<0.0001
Late after Fontan	R^2							
	p Value							

to pneumococcal infections were reported to be the reasons of unfavorable result [5]. The present study suggests that thrombocytosis may be the other risk factor of morbidity and mortality in patients with asplenia syndrome.

The spleen plays a major role in platelet regulation, as it is the primary site of destruction of platelets [3]. In post-splenectomy patients, platelet count rises steeply with a peak value at 7 to 12 days and usually subsides only for the next 2 to 3 months. Thromboembolic complications reportedly occurred in only 1.6% in patients with these reactive thrombocytosis, compared with 12.4% of patients with primary thrombocytosis [9]. Our study showed that patients with asplenia syndrome have higher platelet counts than other Fontan candidates having spleen throughout the surgical stages. The platelet counts were especially high in infancy, when patients have the possibility of BT shunt occlusion, and after Glenn operation, when they often experience cerebral infarction. It is possible that this persistent thrombocytosis due to asplenia may augment the predisposition to developing thromboembolism in patients with a single ventricle.

Platelet adhesion and aggregation are key early events in the development of thromboembolism. Exposed substances from the subendothelium (collagen, tissue factor, von Willebrand factor, and so on) and increased shear force all lead to platelet activation, adhesion, and finally aggregation, which subsequently may cause luminal obstruction or embolization into the microcirculation [10]. Splenectomized patients are at a different risk of thromboembolism according to the kinds of underlying diseases. Nevertheless, Soyer et al. reported that post-splenectomy platelet counts in patients with portal vein thrombosis were higher than those in patients without thrombosis (804×10^9/L vs. 465×10^9/L) [11]. A recent prospective study demonstrated that platelet count of more than 650×10^9/L was significantly associated with post-splenectomy thromboembolic complications [12]. These reports suggest the importance of platelet count as a risk factor of thromboembolic complications. In the present study, more than 550×10^9/L of the platelet count at the first catheterization was selected as the most potent predictor for thromboembolic complications in the multivariate analysis. We should pay more attention to the platelet counts in patients with asplenia syndrome, especially when the platelet count is more than 550×10^9/L at the first cardiac catheterization.

Table 3
Univariate and multivariate analyses of factors influencing thromboembolic complications.

Variable	No. (%)	Odds ratio (95%CI)	*p* Value	
			Univariate	Multivariate
Platelet count > 550×10^9/L [a]	32 (20)	3.17 (1.18–8.30)	0.023	0.046
BT shunt	94 (58)	2.47 (1.02–6.62)	0.044	0.077
Moderate to severe AVVR[a]	32 (20)	1.85 (0.51–6.12)	0.132	0.365
Age > 7y	80 (50)	1.72 (0.76–4.04)	0.178	0.607
Birth weight < 2500 g	26 (16)	1.59 (0.42–5.10)	0.468	0.188
APTT < 40 s [a]	72 (45)	1.29 (0.27–6.02)	0.743	0.166
Systemic ventricular EF < 50% [a]	71 (44)	1.15 (0.19–5.17)	0.839	0.370
Pulmonary vein obstruction	11 (7)	1.11 (0.16–4.64)	0.898	0.760
Hemoglobin > 15 g/dL[a]	37 (23)	1.05 (0.27–3.35)	0.932	0.841
BT shunt size < 3.5 mm [b]	21 (22)	1.68 (0.53–5.01)	0.367	–

BT shunt: Blalock–Taussig shunt, AVVR: atrioventricular valve regurgitation, APTT: activated partial thromboplastin time, EF: ejection fraction.
[a] At the first catheterization.
[b] BT shunt size was investigated among all patients having BT shunt (n = 94).

Our result also showed a significantly higher incidence of thromboembolic complications in patients with asplenia syndrome than in patients with other functionally single ventricles. Patients with asplenia syndrome are often associated with pulmonary vein obstruction. They occasionally underwent a smaller size of the Blalock–Taussig shunt because inherent atrioventricular valve regurgitation may worsen with too much pulmonary blood flow. It seems possible that this pulmonary vein obstruction or smaller shunt size may be another predisposing factor to thromboembolic complications. However, our results showed that only platelet count at the first cardiac catheterization was significantly associated with thromboembolic complications. This result emphasized the clinical importance of elevated platelet count in patients with asplenia syndrome during the treatment course. Antithrombotic therapy should be optimized in patients with asplenia syndrome showing high platelet count.

The limitation of the present study was the lacked assessment of the precise coagulation profile, including protein C, protein S, antithrombin activity, and lupus anticoagulant. Further prospective study is needed to compare the coagulation states in single ventricle patients with and without asplenia syndrome.

6. Conclusion

Our study demonstrated the presence of persistent thrombocytosis as a risk factor of thromboembolism in patients with asplenia syndrome. Precautious monitoring of the platelet count and aggressive antithrombotic or anticoagulation therapy for patients with thrombocytosis

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may be beneficial to decrease the incidence of thromboembolic complications in patients with asplenia syndrome.

Acknowledgments

We thank Junji Kishimoto (Digital Medicine Initiative, Kyushu University Hospital, Fukuoka Japan) and Dr. Masataka Ishimura (Departments of Pediatrics, Graduate School of Medical Sciences, Kyushu University) for the contribution of statistical analysis. The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [20].

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