が起こっている局所からの有害物質の拡散を防止するための生体防御反応と考えられる．以前から血管内に生じた血栓は本来とは異なる部位に生じた病的血栓であるとの認識があったが，上述した血栓は血管内血栓であるにもかかわらず生体防御の役割を果たす生理的血栓と捉えることができる．最近，このようなNETs関与血栓を免疫血栓(immunothrombosis)と呼ぶようになってきており，血管内血栓のすべてが病的血栓とは言えなくなってきた[18]．この免疫血栓の形成反応が過剰になると生理的反応を超えて症候性DVTを発症すると考えることができる[18-20]．動脈血栓はプラークの破綻による内皮損傷部位での血栓であり，血管損傷部位に作られる生理的止血血栓に近い機序によって形成されると考えられるが，動脈血栓の末梢側では血流がうっ滞するために静脈血栓と同様の機序が働いて最終的に動脈を完全閉塞するときの血栓は静脈血栓と似た機序で形成されるのかも知れない[21]．従来，血栓は血小板と血液凝固が主役を演じていると思われていたが，実は白血球，血小板，血液凝固の3者が見事な連携プレーを演じることによって作り出されている．

新たな血栓形成機序からみてどのような抗血栓薬が有用になるのか？

1)FXII阻害薬

現在使われている抗凝固薬の作用機序をみてみると，ワルファリンは第II，VII，IX，X因子を低下させる薬剤であり，ヘパリン類や最近開発された新規経口抗凝固薬(NOAC)は活性化第X因子やトロンビンを阻害する薬剤である．これらの薬剤は静脈血栓開始シグナルとなる単球由来組織因子による凝固反応を阻害することができる．そのためDVTでの有効性が期待でき，実際にDVT再発予防に高い有効性が証明されている．しかしながら，この凝固反応は止血血栓の形成にとっても必須であり，これらの薬剤が出血という有害事象を伴うことは容易に理解できる．しかし，新たな血栓形成機序ではFXIIから始まる内因系凝固が重要な働きをしている(**Fig. 4**)．FXIIは止血血栓の形成に必須ではなく，実際，先天性FXII欠損症の患者は何らの出血傾向も呈さない[17]．また，FXIIノックアウトマウスでは出血時間は正常であるが種々の刺激で誘発した血管内血栓の形成が阻害されていた[1,22,23]．これらのことからFXII阻害薬は出血を起こすことのない抗血栓薬になるのではないかと推測される．

H-D-Pro-Phe-Arg-choromethylketone(PCK)はFXIIを阻害する試薬であるが，PCKを一過性中大脳動脈閉塞マウスに投与しておくと出血時間は全く延長せずに脳梗塞に陥る範囲が狭められた[14,22]．また，塩化鉄による血管内皮損傷モデルマウスでも血栓形成が強く抑制された[14]．

Infestin-4は吸血昆虫の腸に存在するFXII阻害物質であり，吸った血が腸内で固まらないように作用していると考えられ，FXIIインヒビターは自然界で利用されていることになる．現在，infestin-4を薬として開発する研究が進められており[24,25]，一過性中大脳動脈閉塞マウスへの投与で脳梗塞の範囲は小さくなり，神経学的後遺症も少なくなった．一方，出血時間は全く延長しなかった．さらに抗FXIIモノクローナル抗体の開発も進められており，ヒヒを用いた実験で有用性が確認されたことから哺乳類においてもマウス同様の効果が期待される[26]．

2)抗NETs薬

NETsは血栓を強力に促進する作用を有していたことから抗NETs薬は有望な抗血栓薬になりうる．DNaseはNETsの主成分であるDNAを分解するが，DNase Iは嚢胞性線維症患者の去痰を促す吸入治療薬として保険適用になっている(商品名プルモザイム)．この薬を静脈血栓モデルマウスに静注するとNETs形成はほぼ完全に阻害され，血栓も形成されなくなった[1,27]．したがって，DNase Iは強力な抗血栓薬になる可能性がある．また，NETs中のDNAはヒストンと結合しているが，ヘパリンはこの結合を競合的に阻害する作用があり，NETsを不安定化するためNETs阻害薬として機能することが判明した[1,12]．すなわち，ヘパリンは抗凝固作用に加えて抗NETs作用を併せ持つ薬剤といえる．この抗NETs作用はヘパリンの持つ陰性荷電に依存しており，ワルファリンやNOACにはみられない機序である．

3)血栓溶解薬

血栓溶解薬として組織プラスミノーゲンアクチベーター(tPA)が用いられているが，静脈血栓に対す

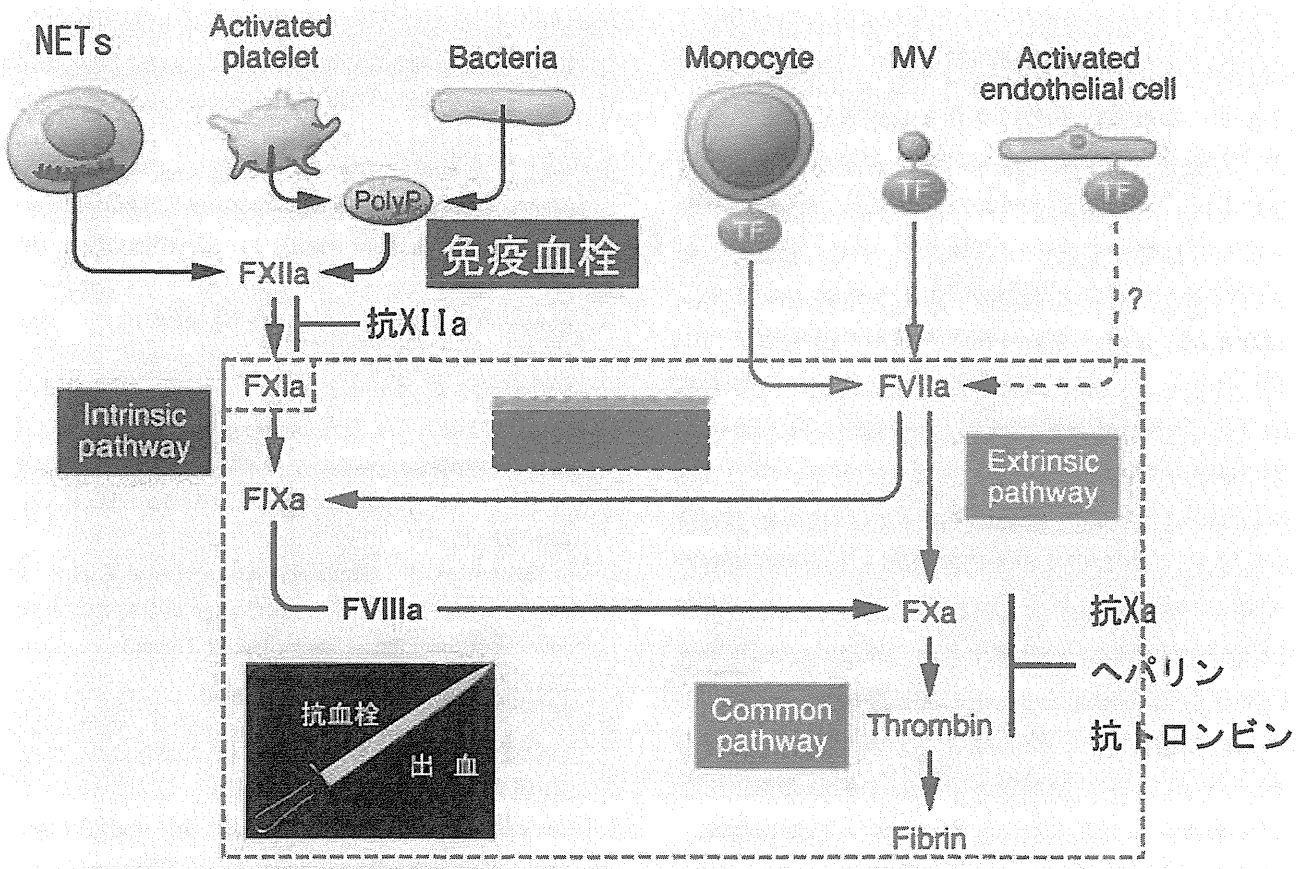


Fig. 4　Activation of the coagulation cascade. NETs activate FXII that is not involved in hemostatic plug but immunothrombus formation. FXII inhibition may offer a selective and safe strategy for preventing DVT. Adapted and modified from reference 2).

る効果には限界のあることが知られている．これは静脈血栓がtPAによって分解可能なフィブリンだけでなくNETsに富んでいるという新しい知見を考えるとむしろ当然と理解できる．まだin vitroの実験ではあるが，血栓の溶解効果はtPAもしくはDNase単独では不十分であるが両者を併用すると完全に溶解したとの結果が得られている[12]．将来の血栓溶解療法はtPAとDNase Iの併用療法が主体になってくるかもしれない．

4)抗血小板薬

静脈血栓形成における血小板の役割は血小板凝集塊の形成よりも白血球を集積させることとNETs形成のトリガーとして働いていることであった．したがって，静脈血栓に対する抗血小板薬は血小板凝集抑制作用よりも抗白血球作用の方が重要であるということになる．アスピリンは血小板凝集抑制効果を有する薬剤として古くから用いられているが，最近，in vivoで抗NETs作用を併せ持っていることが見出された[28]．NETsは炎症下に形成されるが，抗NETs作用が単にアスピリンの抗炎症作用に起因するものではないようで，強力な抗炎症作用を有する副腎皮質ステロイド剤に抗NETs作用は全くなかった．アスピリンの抗NETs作用は通常投与量では現れず，血小板凝集抑制機序とは全く別の機序が推定されている．最近，DVT患者に対して抗凝固療法を6～18カ月継続した後，アスピリンもしくはプラセボを2年間投与してDVTの再発率をみた臨床試験が報告され，アスピリン群の方が有意に少ない再発率を示した[29]．この臨床効果がアスピリンの抗NETs効果と関連しているのかどうかは不明であるが，抗血小板薬も静脈血栓の再発予防に一定の効果を発揮できると考えられ，今後，血小板凝集抑制作用とは違った観点から抗血小板薬の開発が進むかもしれない．

おわりに

新たな静脈血栓モデルマウスの詳細な解析によって，白血球，血小板，凝固の各反応が協調して血栓を作り上げている姿が明らかになった．FXII は生理的な止血血栓の形成には不要だが，DVT の基盤となる免疫血栓の形成には必要であるため FXII 阻害薬は出血を起こさない抗血栓薬となる可能性を秘めている．しかしながら，残された課題はまだたくさんある．ヒト DVT の発症には NETs が関与していそうだが，その血栓がここに示したマウスと同じ機序で作られているとの確証はない[30]．また，FXII 阻害薬は血栓を脆弱・不安定化する可能性があるので肺塞栓を誘発しやすくするかもしれない．また，FXII 阻害薬は免疫血栓の形成を阻害するので感染症の増悪をきたすかもしれない．これらの問題を慎重に検討していく必要はあるが，夢の薬と思われてきた出血性副作用の全くない抗血栓薬を，われわれは本当に手に入れることができるのかという長年の疑問に解答できる時期が，今まさに迫ってきているように思えてならない．

利益相反

本論文発表内容に関連してとくに申告なし．

文　献

1) von Brühl ML, Stark K, Steinhart A, et al: Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice in vivo. J Exp Med 2012; **209**: 819–835
2) Mackman N: New insights into the mechanisms of venous thrombosis. J Clin Invest 2012; **122**: 2331–2336
3) Brill A, Fuchs TA, Chauhan AK, et al: von Willebrand factor-mediated platelet adhesion is critical for deep vein thrombosis in mouse models. Blood 2011; **117**: 1400–1407
4) Simon DI, Chen Z, Xu H, et al: Platelet glycoprotein ibalpha is a counterreceptor for the leukocyte integrin Mac-1 (CD11b/CD18). J Exp Med 2000; **192**: 193–204
5) Brinkmann V, Reichard U, Goosmann C, et al: Neutrophil extracellular traps kill bacteria. Science 2004; **303**: 1532–1535
6) Papayannopoulos V, Zychlinsky A: NETs: a new strategy for using old weapons. Trends Immunol 2009; **30**: 513–521
7) Clark SR, Ma AC, Tavener SA, et al: Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. Nat Med 2007; **13**: 463–469
8) Hakkim A, Fürnrohr BG, Amann K, et al: Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. Proc Natl Acad Sci USA 2010; **107**: 9813–9818
9) Kessenbrock K, Krumbholz M, Schönermarck U, et al: Netting neutrophils in autoimmune small-vessel vasculitis. Nat Med 2009; **15**: 623–625
10) Diaz JA, Fuchs TA, Jackson TO, et al: Plasma DNA is elevated in patients with deep vein thrombosis. J Vasc Surg Venous Lymphat Disord 2013; **1**: doi: 10.1016/j.jvsv.2012.12.002.
11) van Montfoort ML, Stephan F, Lauw MN, et al: Circulating nucleosomes and neutrophil activation as risk factors for deep vein thrombosis. Arterioscler Thromb Vasc Biol 2013; **33**: 147–151
12) Fuchs TA, Brill A, Duerschmied D, et al: Extracellular DNA traps promote thrombosis. Proc Natl Acad Sci USA 2010; **107**: 15880–15885
13) Semeraro F, Ammollo CT, Morrissey JH, et al: Extracellular histones promote thrombin generation through platelet-dependent mechanisms: involvement of platelet TLR2 and TLR4. Blood 2011; **118**: 1952–1961
14) Massberg S, Grahl L, von Bruehl ML, et al: Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. Nat Med 2010; **16**: 887–896
15) Ammollo CT, Semeraro F, Xu J, et al: Extracellular histones increase plasma thrombin generation by impairing thrombomodulin-dependent protein C activation. J Thromb Haemost 2011; **9**: 1795–1803
16) Martinod K, Wagner DD: Thrombosis: tangled up in NETs. Blood 2014; **123**: 2768–2776
17) Renné T, Schmaier AH, Nickel KF, et al: In vivo roles of factor XII. Blood 2012; **120**: 4296–4303
18) Engelmann B, Massberg S: Thrombosis as an intravascular effector of innate immunity. Nat Rev Immunol 2013; **13**: 34–45
19) Schulz C, Engelmann B, Massberg S: Crossroads of coagulation and innate immunity: the case of deep vein thrombosis. J Thromb Haemost 2013; **11** (Suppl 1): 233–241
20) Borissoff JI, ten Cate H: From neutrophil extracellular traps release to thrombosis: an overshooting host-defense mechanism? J Thromb Haemost 2011; **9**: 1791–1794
21) Jackson SP: Arterial thrombosis--insidious, unpredictable and deadly. Nat Med 2011; **17**: 1423–1436
22) Kleinschnitz C, Stoll G, Bendszus M, et al: Targeting

coagulation factor XII provides protection from pathological thrombosis in cerebral ischemia without interfering with hemostasis. J Exp Med 2006; **203**: 513–518
23) Renné T, Pozgajová M, Grüner S, et al: Defective thrombus formation in mice lacking coagulation factor XII. J Exp Med 2005; **202**: 271–281
24) Hagedorn I, Schmidbauer S, Pleines I, et al: Factor XIIa inhibitor recombinant human albumin Infestin-4 abolishes occlusive arterial thrombus formation without affecting bleeding. Circulation 2010; **121**: 1510–1517
25) Xu Y, Cai TQ, Castriota G, et al: Factor XIIa inhibition by Infestin-4: in vitro mode of action and in vivo antithrombotic benefit. Thromb Haemost 2014; **111**: 694–704
26) Matafonov A, Leung PY, Gailani AE, et al: Factor XII inhibition reduces thrombus formation in a primate thrombosis model. Blood 2014; **123**: 1739–1746
27) Brill A, Fuchs TA, Savchenko AS, et al: Neutrophil extracellular traps promote deep vein thrombosis in mice. J Thromb Haemost 2012; **10**: 136–144
28) Lapponi MJ, Carestia A, Landoni VI, et al: Regulation of neutrophil extracellular trap formation by anti-inflammatory drugs. J Pharmacol Exp Ther 2013; **345**: 430–437
29) Becattini C, Agnelli G, Schenone A, et al: Aspirin for preventing the recurrence of venous thromboembolism. N Engl J Med 2012; **366**: 1959–1967
30) Savchenko AS, Martinod K, Seidman MA, et al: Neutrophil extracellular traps form predominantly during the organizing stage of human venous thromboembolism development. J Thromb Haemost 2014; **12**: 860–870

Abstract

Evaluation of Anti-thrombotic Drugs in View of Recent Mechanism for Venous Thrombus Formation

Takaaki Hato

Division of Transfusion Medicine and Cell Therapy, Ehime University Hospital

Key words: venous thrombosis, neutrophil, monocyte, platelets, factor XII

Recent studies established a novel mouse model of deep vein thrombosis (DVT) induced by flow restriction, closely resembling the time course and histological features of DVT in humans. Detailed analysis of this model revealed that venous thrombi were formed by more elegant biological reactions than expected. In this model, the compromised venous blood flow induced endothelial cell activation initiating expression of adhesive ligands and generation of inflammatory cytokines, resulting in recruitment of monocytes, neutrophils, and platelets. Recruited monocytes trigger the extrinsic coagulation cascade via monocyte-derived tissue factor. Neutrophils released their nuclear substances decorated with granular proteins (NETs) which in turn trigger the FXII-initiated intrinsic coagulation cascade. Platelets support leukocyte accumulation and strongly promote NETs formation. These findings suggest that inhibition of FXII and NETs may be promising strategies for the treatment of DVT. Unraveling the molecular mechanism of DVT would help better understanding of the characteristics of current anti-thrombotic drugs and pave the way to the development of novel anti-thrombotic drugs with selective and safe profiles.

◆症例報告◆ 脳卒中37: 36–40, 2015

Crescendo TIAを呈したJAK2変異陽性本態性血小板血症の1例

岡田 陽子[1] 加藤 丈陽[1] 山下 泰治[1] 越智 雅之[1] 永井 勅久[1]
越智 博文[1] 伊賀瀬道也[1] 羽藤 高明[2] 小原 克彦[1]

要旨：症例は53歳男性，右上肢の感覚障害と脱力発作を繰り返し，脳梗塞を指摘され入院した．本態性血小板血症と診断され，JAK2遺伝子変異を有した．入院後も右上肢の脱力症状を繰り返し，急性期には低容量アスピリンと抗凝固薬の併用を要したが，骨髄抑制療法の効果発現後にはアスピリンが中止可能となった．JAK2遺伝子変異陽性例では，血小板数や機能の異常のみでなく，凝固因子の異常や血管内皮の機能障害を認めることが指摘されており，高齢，血栓塞栓症の既往などに加えて，本態性血小板血症における血栓塞栓症のリスク因子の一つであるといわれる．本症例はアテローム血栓性脳梗塞に類似した臨床経過を呈し，血管内皮の機能障害を背景とした血管壁の血栓易形成性が，脳梗塞発症に関連している可能性を推測した．本疾患における最適な抗血栓療法を検討する上で貴重な症例であると考えられ，報告する．

Key words: essential thrombocythemia, JAK2 gene, thrombosis, antiplatelet agents, anticoagulant

はじめに

本態性血小板血症(ET)は，骨髄増殖性疾患の一つであり，脳梗塞を含む血栓塞栓症は予防すべき重要な合併症である．しかしその頻度は，脳梗塞全体からみると1%未満と低く[1]，その脳梗塞の発症機序や病態はまだ十分解明されていない．今回我々は，JAK2遺伝子変異を有するETを背景に，TIA発作を反復した後に脳梗塞を発症した1例を経験した．骨髄抑制療法に加えて，急性期には低容量アスピリンと抗凝固薬の併用療法が有効であった．本症例における病態，治療に関する文献的考察を加え報告する．

症　例

症例：53歳，男性
主訴：右手の感覚障害
家族歴：特記すべき事項なし．
既往歴：53歳時　高血圧
現病歴：X年2月某日，急にネクタイの締め方がわからなくなり，A病院を受診したが，症状は速やかに消失したため経過観察となった．

同年8月頃，急に右手にジンジン感が出現したが，1時間程度で完全に消失した．その後も30分から1時間程度持続する右上肢の異常感覚が，徐々に症状の強さや頻度を増しながら出現するようになり，同時に右上肢の脱力感も自覚するようになった．同年12月の発作時には右手でものをつかむことができなくなり，B病院を受診し，頭部MRIで左中大脳動脈領域に散在性の拡散強調画像(DWI)高信号病変を指摘され，脳梗塞と診断され入院した．MRAでは左中大脳動脈に壁不整を認め，FLAIR画像では対側に陳旧性病変を認めた(Fig. 1)．両側散在性病変を認めることから塞栓症を疑われ，未分画ヘパリン1万単位/日の持続投与が開始されたが，入院翌日には，右上肢の感覚障害と麻痺が増悪し，加えて失語も出現した．DWIで一部新たな病変や既知の病変の拡大を認めたため，ヘパリンを1万5千単位/日に増量し，アスピリンの内服が追加された．症状は改善傾向となったが，第3病日にヘパリンを減量したところ右上肢の感覚障害が再燃し，1万2千単位に再度増量された．脳梗塞の原因が確定せず，第14病日に当院に転院した．転院後ヘパリンからワルファリン内服に切り替え，アスピリンは中止した．ワルファリンはINR 1.6〜2.6を

[1]愛媛大学医学部附属病院老年・神経・総合診療内科学
[2]同　血液・免疫・感染症内科学
(2014年5月7日受付，2014年6月23日受理)

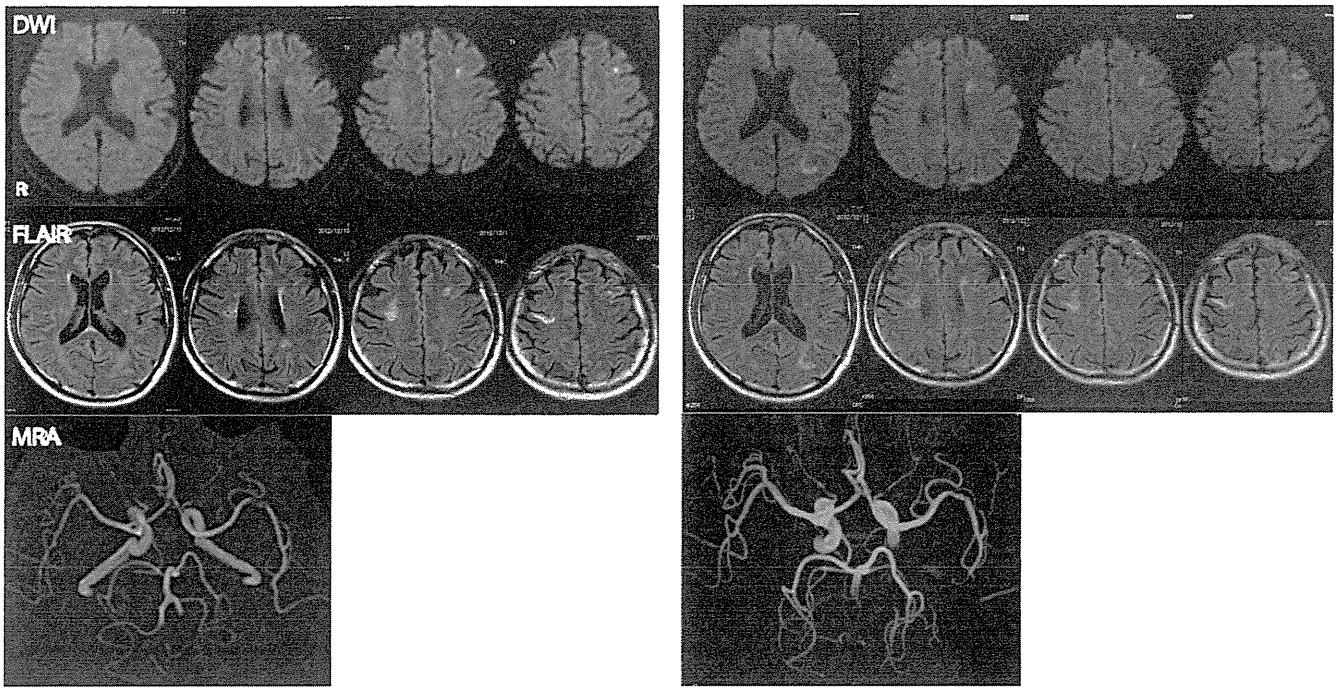


Fig. 1

A：B 病院入院時頭部 MRI
DWI で左 MCA 領域に散在性の高信号病変を認め，FLAIR では対側にも高信号病変を認める．MRA では左 MCA の壁不整を認める．
B：入院翌日
DWI で一部高信号病変の拡大と新規虚血病変を認める．

目標にコントロールを行い，内服開始約 1 週間程度で治療域となった．4 日後に右上肢の感覚障害と麻痺が出現したため，アスピリンを再開した．数年前から血小板増多を指摘されており，前医入院時にも血小板増多を認め（Table 1），当院血液内科を受診し，ET と診断され，JAK2 遺伝子 V617F 変異を有することが判明した．血液検査では血小板増多以外に脳梗塞に関連した異常所見はなく，その他の検査でも脳梗塞の原因となり得る異常を認めず，ET による脳梗塞と診断した．第 35 病日よりヒドロキシカルバミドの内服を開始し，第 36 病日にアスピリンを中止し，抗血栓薬はワルファリン単剤とした．しかし，第 41 病日に右上肢の感覚障害が再燃したため，出血の危険因子となる後天性 von Willebrand 症候群がないことを確認した上で，アスピリンとワルファリンの併用療法を再開した．第 49 病日に自宅退院し，6 カ月後に血小板数の正常化を確認した上でアスピリンの併用を中止し（Fig. 2），現在まで 1 年以上再発は認めていない．

考　察

ET は，多能性幹細胞の腫瘍性増殖により骨髄巨核球の過形成を来し，血小板増加をもたらす疾患である．新 WHO 分類では慢性骨髄性白血病，原発性骨髄線維症，真性多血症とともに慢性骨髄増殖性疾患に分類される[2]．ET では，13％の症例で血栓塞栓症を合併し，その年間再発率は 5.6％と報告されている[3]．ET を含む骨髄増殖性疾患に伴う脳梗塞の頻度は，脳梗塞全体の 1％未満[1]と高くはないが，診断した際には血栓塞栓症に対する適切な一次および二次予防が必要である．

ET における血栓塞栓症の危険因子として，60 歳以上の高齢，血栓塞栓症の既往[4,5]，などが報告されている．血小板数に関しては，150 万 /μL 以上[6]あるいは 60 万 /μL 以下のいずれもリスクになるとの報告があり一定しない．近年，これらに加えて JAK2 遺伝子変異が血栓塞栓症の危険因子として報告されている．JAK2 遺伝子変異は，真性多血症の 95％，ET の約半数で認められ，真性多血症ではそのほとんどが変異遺伝子のホモであるのに対して，ET ではヘテロが多数を占める．この変異を有する場合，ET の血栓塞栓症リスクは約 2 倍となることが報告されている[7]．しかしながら，遺伝子変異の有無によって血栓塞栓症の病型やその後の経過に違いがあるかどうかに関しては，まだ十分な検討はない．

Table 1　B病院入院時の血液検査所見

血液検査(B病院入院時)			
(血液検査)		(血液生化学検査)	
WBC	7190/μl	TP	6.9 g/dl
Neu	63.8%	Alb	4.5 g/dl
Lym	25.7%	T.Bil	0.8 mg/dl
Eosino	2.4%	ChE	409 U/L
Mono	6.2%	AST	20 U/L
Baso	1.9%	ALT	28 U/L
RBC	556×10^4/μl	LDH	253 U/L
Hb	17.8 g/dl	ALP	282 U/L
Hct	53.5%	γ-GTP	76 U/L
MCV	96.2 fl	UA	6.1 mg/dl
MCH	32.0 pg	TG	91 mg/dl
MCHC	33.3 g/dl	HDL-C	56 mg/dl
PLT	65.4×10^4/μl	LDL-C	83 mEq/L
(凝固系)		BUN	12.8 mg/dl
APTT	44.1 sec	Cre	0.88 mg/dl
PT	102.7%	Na	138 mEq/L
PT-INR	0.94	K	5.9 mEq/L
D-dimer	0.4 μg/ml	Cl	103 mEq/L
		CRP	0.05 mg/dl
		Glu	104 mg/dl
		HbA1c	5.1%

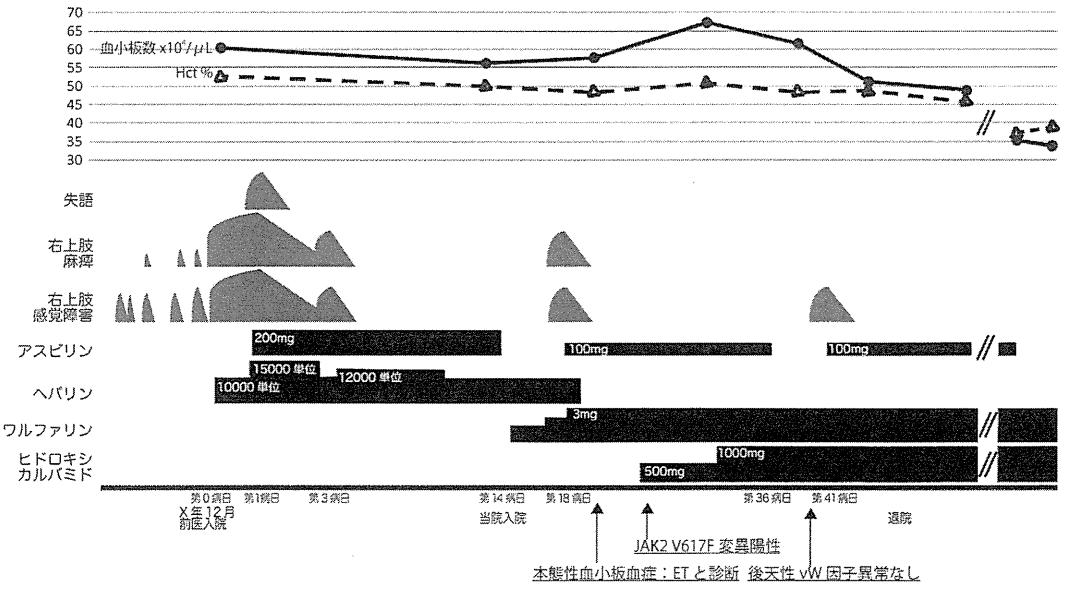


Fig. 2　入院後の経過

JAK2遺伝子変異陽性例において血栓塞栓症のリスクが高い理由については，様々な検討がなされている．血小板自体の機能異常や，凝固因子および凝固阻止因子の異常に加えて，JAK2遺伝子変異陽性例では，可溶性トロンボモジュリンや可溶性セクレチンが上昇しており，血管内皮の抗血栓作用が低下していることが指摘されている[8]．本症例と同様に中大脳動脈の壁不整を認めた症例報告において，狭窄部位が経過観察中に移動したこと

が報告されており，血管内皮の機能障害に伴う血管壁の血栓易形成性が病態の一つとして推測されている[9]．本症例では，約半年間，同一の症状が繰り返し程度を増しながら出現しており，中大脳動脈遠位部の壁在血栓による血栓症の可能性が考えられた．

ETの高リスク例では骨髄抑制療法に加えて抗血栓薬の投与が推奨され，抗血栓薬としては一般的にアスピリンが使用される[2,4]．低容量アスピリンの使用は，ETにおける血栓塞栓症を減少させることが確認されており[10]，本症例でもアスピリン投与の有無に伴う症状の変動を認めている．また，ヒドロキシカルバミドも，高リスクのET患者における血栓塞栓症を減少させることが知られており[6]，本症例において血小板数が正常化した後にはアスピリンの中止が可能となった．これらのことは，現在推奨されている，高リスク例での骨髄抑制療法および低容量アスピリン投与という治療方針が妥当であることを示している．

しかし，真性多血症およびETそれぞれ2000例弱を対象として，使用薬剤と血栓塞栓症の関連を後向きに検討した報告では，抗血小板薬と抗凝固薬との間に血栓塞栓症の再発率には有意差はなかった．血栓塞栓症の内訳別の検討では，脳梗塞に対しては抗血小板薬が，静脈系血栓症に対しては抗凝固薬の方が，再発予防効果が高く，また，出血性合併症は抗凝固薬使用例でわずかに多いものの有意差は認めなかった[3]．ETにおける血栓塞栓症は，本症例のような脳梗塞や心筋梗塞などの動脈性血栓が60～70％であり，残りは深部静脈血栓症やBudd-Chiari症候群などの静脈血栓症が占めるといわれている[3]．Santilliら[8]は，真性多血症患者において，血管内皮の修復機能低下やNO産生の低下に加えて，アスピリン抵抗性のトロンボキサン合成増加が認められることを報告しており，低容量アスピリン以外の抗血栓薬を使用する必要性を述べている．本症例においても，前医ではヘパリン減量時に症状の悪化を認めており，現在もワルファリンを継続し再発を認めていないことから，抗凝固薬の有効性についても今後十分検討されるべきである．また，血小板薬についても，アスピリン以外の薬剤，特に内皮機能の改善効果を有するシロスタゾールや，2剤併用療法が有効である可能性が考えられるが，これらに関する検討はまだない．

ETにおける脳梗塞において，JAK2遺伝子変異陽性例および陰性例での臨床経過の違いや抗血栓薬の比較検討に関して，今後の症例蓄積により，さらに重要な知見が得られるものと考える．

結　語

JAK2遺伝子変異を有する本態性血小板血症を背景に，反復するTIA発作に続き脳梗塞を発症した症例を経験した．アスピリンの中止により症状の再燃を繰り返したが，骨髄抑制療法の効果発現後には，中止可能となり，本疾患における骨髄抑制療法および低容量アスピリンの有用性が確認された．しかしながら，静脈系血栓症例，血管内皮機能障害や凝固異常の存在例などにおける，最適な抗血栓療法については，抗凝固療法を含めて今後も検討する必要があると考える．

本論文の要旨は第39回日本脳卒中学会総会で発表した．

本論文に関連し，開示すべきCOIはありません．

参考文献

1) Gonthier A, Bogousslavsky J: Cerebral infarction of arterial origin and haematological causation: the Lausanne experience and a review of the literature. Rev Neurol (Paris) 160: 1029–1039, 2004
2) 柏木浩和：本態性血小板血症の診断と治療．血栓止血誌 19: 4, 2008
3) De Stefano V, Za T, Rossi E, et al: Recurrent thrombosis in patients with polycythemia vera and essential thrombocythemia: incidence, risk factors, and effect of treatments. Haematologica 93: 372–380, 2008
4) Vannucchi AM: Insights into the pathogenesis and management of thrombosis in polycythemia vera and essential thrombocythemia. Intern Emerg Med 5: 177–184, 2010
5) Tefferi A: Polycythemia vera and essential thrombocythemia: 2012 update on diagnosis, risk stratification, and management. Am J Hematol 87: 285–293, 2012
6) Birgegård G: Long-term management of thrombocytosis in essential thrombocythaemia. Ann Hematol 88: 1–10, 2009
7) Lussana F, Caberlon S, Pagani C, et al: Association of V617F Jak2 mutation with the risk of thrombosis among patients with essential thrombocythaemia or idiopathic myelofibrosis: a systematic review. Thromb Res 124: 409–417, 2009
8) Santilli F, Romano M, Recchiuti A, et al: Circulating endothelial progenitor cells and residual in vivo thromboxane biosynthesis in low-dose aspirin-treated polycythemia vera patients. Blood 112: 1085–1090, 2008
9) Naganuma M, Isoda K, Nishi S, et al: Repeated episodes of ischemic stroke over a short period in a patient with essential thrombocythemia on anticoagulant therapy. J Stroke Cerebrovasc Dis 23: 166–168, 2014
10) Squizzato A, Romualdi E, Passamonti F, et al: Antiplatelet drugs for polycythaemia vera and essential thrombocythaemia. Cochrane Database Syst Rev 4: CD006503, 2013

Abstract

A case of ischemic stroke after recurrent transient ischemic attacks due to essential thrombocythemia with JAK2 mutation

Yoko Okada, M.D., Ph.D.,[1] Takeaki Kato, M.D.,[1] Taiji Yamashita, M.D.,[1] Masayuki Ochi, M.D., Ph.D.,[1] Tokihisa Nagai, M.D., Ph.D.,[1] Hirofumi Ochi, M.D., Ph.D.,[1] Michiya Igase, M.D., Ph.D.,[1] Takaaki Hato, M.D., Ph.D.,[2] and Katsuhiko Kohara, M.D., Ph.D.[1]

[1]Department of Neurology and Geriatric Medicine, Ehime University Graduate School of Medicine
[2]First Department of Internal Medicine, Ehime University Graduate School of Medicine

Essential thrombocythemia (ET) is characterized by thrombocytosis with an increased risk of thromboembolism, and is a rare cause of stroke. Approximately half of the patients with ET have JAK2V617F mutation, and these patients have double the risk of thromboembolism compared to ET patients without the mutation. Strategies for the management of stroke in patients with ET, especially those with JAK2 gene mutation, are not well established. We describe a 53-year-old patient with ET and JAK2V617F mutation who presented with stroke after recurrent transient ischemic attacks. Magnetic resonance imaging showed scattered ischemic lesions in the territory of the left middle cerebral artery, and magnetic resonance angiography showed stenosis of the left middle cerebral artery. The patient was treated with aspirin and warfarin. Several early attempts to withdraw either aspirin or warfarin resulted in deterioration of his right arm numbness. He was prescribed hydroxycarbamide by a hematologist and his platelet count normalized after 6 months. Aspirin was then successfully withdrawn, with no further signs of cerebral ischemia during the following year. Endothelial dysfunction has been proposed as one of the causes of thromboembolism in patients with JAK2V617F mutation, in addition to platelet activation. Our experience suggests that patients with ET and JAK2V617F mutation may benefit from combined antiplatelet and anticoagulant therapy in acute phase.

Key words: essential thrombocythemia, JAK2 gene, thrombosis, antiplatelet agents, anticoagulant

(Jpn J Stroke 37: 36–40, 2015)

血小板部会
「抗血小板薬の分子標的とそのリスク・ベネフィット」

部会長　羽藤　高明（愛媛大学　輸血・細胞治療部）

血小板部会では「抗血小板薬の分子標的とそのリスク・ベネフィット」をテーマに取り上げた．今回対象とした抗血小板薬はアスピリン，クロピドグレル，シロスタゾールの代表的抗血小板薬と，本邦では未発売ながら海外で使用されている GPIIb-IIIa 阻害薬と PAR-1 阻害薬の計 5 製剤である．それぞれの薬剤の標的分子からみた作用と投与患者におけるリスク・ベネフィットという基礎・臨床両面からの報告があり，活発な討議が行われた．

まず，東北大学の堀内久徳先生に今回のテーマについての総括レビューをしていただいた．血小板活性化経路は複雑で様々なルートからのシグナルが協調して血小板の活性化が導かれており，この活性化経路内のどの分子に抗血小板薬が作用しているのかという全体像が示された．臨床的に抗血小板薬は脳梗塞・心筋梗塞の再発予防に用いられている．とくに，冠動脈ステント療法を受けた患者では最も重篤な合併症であるステント血栓症を予防するために抗血小板薬は欠くことのできない薬剤となっており，抗血小板薬なしには行うことのできない医療が存在する時代になっている．一方，非ステント患者での有効性は 20％程度の再発抑制にとどまっているのが現状であり，さらなる改善が望まれている．一方，これらの効果は薬理作用から明らかなように出血性合併症というリスクを伴うことは容易に理解される．近年開発されてきた抗血小板薬は強力な抗血小板作用を有するが，その投与量によってベネフィットとリスクの比が大きく変わることがわかり，至適用量の設定が焦点になってきていることが報告された．

慶應大学の松原由美子先生には cyclo-oxygenase 阻害薬について報告していただいた．その代表的薬剤であるアスピリンは血小板 cyclo-oxygenase を不可逆的に阻害することによって強力な血小板凝集惹起物質であるトロンボキサン A2 の産生を阻害する．しかし，血管内皮細胞が産生するプロスタグランディン I2 の産生も阻害するというマイナス面がある．また，アスピリンはアラキドン酸代謝経路をリポキシゲナーゼ系にシフトする作用があり，ロイコトリエンなどの代謝に影響をおよぼすことが指摘されている．臨床面では日本人におけるアスピリンの一次予防効果をみた大規模臨床試験が最近報告されたが，その有効性は認められなかった．さらに，アスピリンは消化管出血をはじめとする出血リスクを背負っており，このリスク評価は重要な点である．また，アスピリンのモニタリング法も開発されているが，その意義については議論のある現状が報告された．

三重大学の西川政勝先生には P2Y12 阻害薬について報告していただいた．その代表的薬剤であるクロピドグレルは肝チトクローム酵素（主に CYP2C19）の代謝を介して活性代謝物が生成され，抗血小板作用が発揮される．そのためクロピドグレルの作用は CYP2C19 の遺伝子多型によって影響を受けることが知られており，クロピドグレル低反応性を示す患者群が存在する．近年市販されたプラスグレルは CYP2C19 遺伝子多型の影響を受けにくい薬剤であり，クロピドグレルよりも強力な血小板凝集抑制効果を示す．しかし，その反面，出血性合併症が多いというリスクを有することが欧米の臨床試験で示されてきた．本邦ではプラスグレルの投与量を欧米用量の 1/3 に設定することによって，このリスク・ベネフィット比を向上させることに成功した．P2Y12 阻

害薬の至適投与量を決定することは重要であり，そのための薬効モニタリング法がいくつか開発されてきた．しかしながら，その結果に基づいて投与量を増減しても臨床転帰は何ら変わらないことが判明し，現状でのモニタリング検査実施の意義は確立していないことが報告された．

愛媛大学の山之内純先生にはPDE3阻害薬について報告していただいた．その代表的薬剤であるシロスタゾールはPDE3による血小板内cAMPの分解を抑制してcAMP濃度を上昇させるため血小板凝集を抑制するとともに血管平滑筋を弛緩させることによる血管拡張作用を有する．本剤は出血性副作用が少ないという特徴を有しているもののin vitroでの血小板凝集抑制効果が弱いとされており，通常の血小板凝集能検査でシロスタゾールの薬効を検出することは困難であった．しかし，脳梗塞の再発予防効果は複数の無作為対照試験で証明されており，in vivoでの効果は十分にあると考えられる．最近，シロスタゾールの薬効を鋭敏に検出できる方法が開発された．シロスタゾールのモニタリング検査がどれほどの臨床的意義をもつのかは不明であるが，モニタリング可能な手段を確立できたことが報告された．

大阪大学の冨山佳昭先生にはGPIIb-IIIa阻害薬について報告していただいた．GPIIb-IIIaはすべての血小板凝集において血小板同士を結合させる受容体として機能しているため，その抑制によりすぐれた抗血栓作用が期待される．しかし，その反面，出血性副作用の懸念が大きくなる．静注薬であるため主にPCI患者に投与されてきたが，アスピリン・クロピドグレル併用療法によって一定の成績が得られている現在，欧米では高リスクPCI症例に限定して使用されている．また，経口投与が可能なGPIIb-IIIa阻害薬も開発されたが，大規模臨床試験で逆に心筋梗塞が増加するという結果となり，開発は中止された．その原因として経口GPIIb-IIIa薬が実はアゴニスト作用も有していて，GPIIb-IIIaを活性化したためとの指摘があり，実際にGPIIb-IIIa分子の立体構造が変化して活性化される様子が後に捉えられた．また，GPIIb-IIIaの活性化維持にP2Y12受容体からのシグナルが重要であることがわかり，P2Y12阻害薬は実はGPIIb-IIIaの活性化が持続できなくなることによって抗血栓作用を発揮しているという興味ある検討結果が報告された．

東京女子医大の山崎昌子先生にはPAR-1阻害薬について報告していただいた．PAR-1はトロンビン受容体として同定され，トロンビンはPAR-1受容体を介したシグナルによって強い血小板活性化反応を惹起することから，PAR-1は抗血小板薬の標的分子として注目されてきた．また，抗トロンビン薬と違ってPAR-1阻害薬は凝固機転によるフィブリンの産生を阻害しないので出血性副作用が少なくなることが期待された．しかし，第3相試験では有効性が証明されたものの出血性イベントが有意に増加し，とくに脳卒中既往のある患者で頭蓋内出血が増加する結果となった．このプロファイルから，脳卒中既往患者を適応外とする条件でFDAの承認が得られた．PAR-I阻害薬については有効性と安全性のバランスがとれた投与条件をさらに見出していくことが重要であることが報告された．

以上の6演題の発表と討議から，抗血小板薬を分子標的薬の一つと捉えていく必要性を感じた．とくに，抗血小板薬の標的分子からみた薬理効果と臨床的予測結果が一致しない点は重要で，その解析から課題が見えてきている．それを踏まえて抗血小板薬のリスク・ベネフィット比が最適となる投与条件を見出すことが重要なポイントであると思われた．

Transfusion Medicine and Hemotherapy

Case Report

Transfus Med Hemother 2015;42:59–63
DOI: 10.1159/000370225

Received: December 18, 2013
Accepted: May 19, 2014
Published online: December 19, 2014

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

Yoshiyuki Ogawa[a] Masanori Matsumoto[b] Hisanobu Sadakata[c] Ayami Isonishi[b] Seiji Kato[b] Yoshihisa Nojima[a] Yoshihiro Fujimura[b]

[a] Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine, Maebashi City, Japan;
[b] Department of Blood Transfusion Medicine, Nara Medical University, Kashihara City, Japan;
[c] Department of Obstetrics and Gynecology, Gunma University Graduate School of Medicine, Maebashi City, Japan

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

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

Introduction

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

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

Yoshiyuki Ogawa and Masanori Matsumoto equally contributed in preparing this manuscript.

KARGER

Fax +49 761 4 52 07 14
Information@Karger.com
www.karger.com

1660–3796/15/0421–0059$39.50/0

Accessible online at:
www.karger.com/tmh

Dr. Yoshihiro Fujimura
Department of Blood Transfusion Medicine
Nara Medical University
Shijyo-cho 840, Kashihara City, Nara, 634-8522, Japan
yoshifuji325@naramed-u.ac.jp

Table 1. Plasma levels of anti-ADAMTS13 autoantibodies

Age, years	Gestational weeks	FFP infusion	ADAMTS13 activity, %	ADAMTS13 inhibitor, BU/ml	ADAMTS13 IgG type antibody, units/ml	Clinical status
21	*	–	<0.5	1.4	42.9	TTP bout
22	*	–	<0.5	1.7	35.0	remission
27	*	–	3.7	0.8	48.9	remission
27	*	–	1.9	1.6	33.3	remission
30	8	–	<0.5	<0.5	28.2	pregnancy
30	10	+	6.5	0.5	34.4	pregnancy
30	11	+	4.5	0.5	31.2	pregnancy
30	13	+	3.4	0.5	19.9	pregnancy
30	15	+	3.3	0.8	30.2	pregnancy
30	20	+	3.2	0.9	23.7	pregnancy
30	24	+	2.9	<0.5	21.6	pregnancy
30	29	+	2.3	0.6	13.9	pregnancy
30	33	+	3	<0.5	19.7	pregnancy
30	38	+	2.9	<0.5	14.9	pregnancy
30	39	+	6.9	0.5	16.1	pregnancy
30	*	–	1.9	0.6	13.9	1 month after delivery
32	*	–	5.2	<0.5	15.4	1.5 years after delivery
32	*	–	1.8	<0.5	9.8	2 years after delivery

microangiopathic hemolytic anemia in childhood that are reversible by infusions of fresh frozen plasma (FFP) (early-onset phenotype) [3]. On the other hand, patients with the 'late-onset phenotype' are diagnosed with USS in adulthood, usually during episodes of infectious disease or pregnancy [3]. Moatti-Cohen et al. [4] reported that the rate of USS is much higher in pregnancy-onset TTP patients than in all adulthood-onset TTP patients.

We previously described 43 USS patients in Japan up to the end of March 2011 [3]. Among them, 9 patients developed bouts of TTP and were correctly diagnosed with USS in association with pregnancy [5]. These pregnancies often result in premature delivery or fetal loss. Recent papers have reported successful delivery with FFP infusion therapy in patients with USS diagnosed prior to pregnancy [6, 7]. However, a detailed therapeutic protocol including FFP infusions for pregnant women with USS has not yet been established.

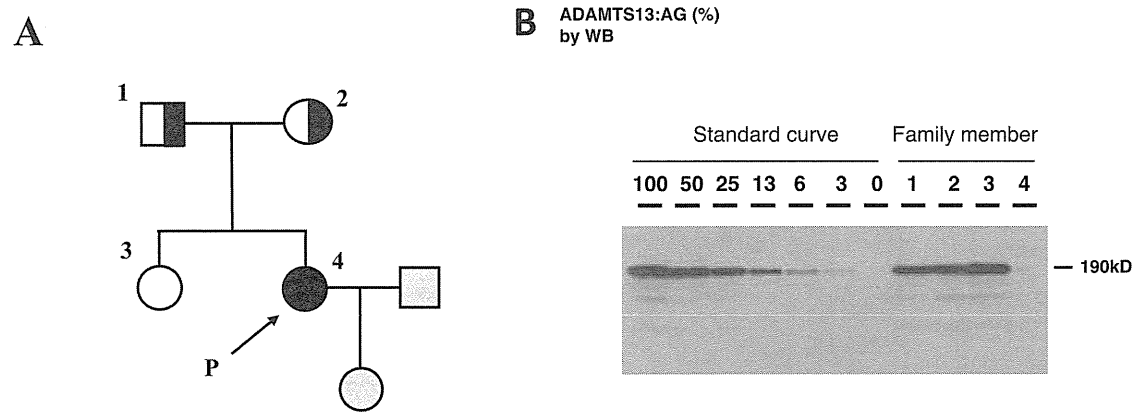
Here, we report a USS patient with low titers of neutralizing (inhibitory) and non-neutralizing (binding) antibodies against ADAMTS13 who successfully underwent delivery with the use of gradually increasing FFP infusions as the pregnancy progressed. The intervals between and volumes of FFP infused were determined by close monitoring of levels of ADAMTS13 activity and its inhibitor.

Material and Methods

Until 2005, ADAMTS13 activity was analyzed by a VWF multimer assay with a detection limit of 3% of normal controls [2, 8]. Since 2005, a highly sensitive chromogenic ADAMTS13-act-ELISA [9] with a detection limit of 0.5% of normal was developed and replaced the VWF multimer assay. Thus, we re-examined ADAMTS13 activity in stored plasma samples using this act-ELISA and reported the results by the act-ELISA in this study. Plasma ADAMTS13 inhibitor titers were also re-examined using the chromogenic ADAMTS13-act-ELISA in heat-inactivated plasma at 56 °C for 30 min. One Bethesda unit (BU) of inhibitor was defined as the amount of inhibitor that reduces ADAMTS13 activity to 50% of control [10]. ADAMTS13 inhibitor titers were defined as: <0.5 BU/ml (negative), 0.5–1.0 BU/ml (marginal), and ≥1.0 BU/ml (positive). Plasma levels of ADAMTS13 antigen were determined using a quantitative sandwich ELISA assay [11]. Plasma ADAMTS13 antigen was also analyzed by quantitative and qualitative western blotting (WB) under reducing conditions [12]. Densitometric analysis of ADAMTS13 antigen was performed for the 190 kDa band using NIH imageJ (developed by the National Institutes of Health, *http://rsb.info.gov/nih-image/*). Plasma anti-ADAMTS13 IgG antibody titers (binding antibody) were determined by TECHNOZYM® ADAMTS-13 INH (Technoclone, Vienna, Austria) according to the manufacturer's instructions. In this assay, plasma IgG levels less than 12 units/ml were defined as negative, 12–15 units/ml were considered borderline, and levels greater than 15 units/ml were defined as positive. *ADAMTS13* gene analyses [13] were performed with the permission of the Ethics Committees. The pathogenicity of missense mutations was analyzed in silico using PolyPhen-2 (*http://genetics.bwh.harvard.edu/pph2/*) to predict the functional significance of missense mutations. Written informed consent for *ADAMTS13* gene analysis was obtained from the patient and her family.

Case Report

Proband LL4 is a female born in 1981. Her parents and elder sister are apparently healthy. She did not have any episodes of severe neonatal jaundice requiring exchange blood transfusion. At 14 years of age, she developed thrombocytopenia and acute renal failure requiring hemodialysis during an upper respiratory tract infection. She had similar episodes during upper respiratory tract infections at the ages of 15, 16, 17, and 20 years. These bouts were ameliorated by FFP infusion. At 21 years of age, she was admitted to a local hospital complaining of diarrhea and high-grade fever. She was diagnosed with TTP based on the pentad of hemolytic anemia, thrombocytopenia, acute renal failure, fever, and mild neurological symptoms. Her condition improved with FFP administration. Soon after this episode, she got married. When the patient was 27 years old, detailed investigation including *ADAMTS13* gene analysis was performed in all members of her family. At 28 years of age, she underwent an elective termination at 6 weeks of gestation after the risk of developing TTP was taken into consideration. She has never received prophylactic FFP infusions without the presence of thrombocytopenia.



C

	ADAMTS13:AC (%)	ADAMTS13:AG (%)		*ADAMTS13 gene*					
	ELISA	ELISA	WB	Thr339	Cys438	Gln448	Pro475	Pro618	Gly909
-1	27	23	37	T/T	C/S	Q/Q	P/P	P/P	G/G
-2	34	50	51	T/R	C/C	Q/E	P/S	P/A	G/R
-3	57	77	97	T/T	C/C	Q/Q	P/S	P/P	G/G
-4	3.7	1.2	<3	T/R	C/S	Q/E	P/P	P/A	G/R

Fig. 1. Pedigree and ADAMTS13 analysis of USS-LL4 and her family. **A** The propositus (denoted as P), USS-LL4, is the second child of nonconsanguineous parents. Squares and circles indicate males and females, respectively, and shaded symbols represent individuals who were not examined. Half-black symbols indicate asymptomatic carriers. **B** WB analyses of plasma ADAMTS13 antigen (AG) in the patient's family members are shown. **C** ADAMTS13 activity (AC) was determined using activity ELISA, and ADAMTS13 AG levels were measured using ELISA and WB. Results are shown as percentages of normal values. Identified mutations in ADAMTS13 are depicted using one-letter amino acid abbreviations.

ADAMTS13 Activity, Antibody, and Antigen Analysis

Plasmas obtained at 21 and 22 years of age showed severely decreased ADAMTS13 activity (<0.5 % of normal) and low titers of ADAMTS13 inhibitor (1.4 and 1.7 BU/ml, respectively) (table 1). In addition, ADAMTS13 binding IgG antibodies were found in both samples. These results indicated that this patient might have acquired TTP or USS with the presence of ADAMTS13 inhibitor. As shown in figure 1C, plasma ADAMTS13 antigen levels as analyzed by ELISA were 1.2% of normal values in the patient at 27 years of age. Further, plasma levels of ADAMTS13 antigen analyzed by WB were <3% of normal values in the patient.

ADAMTS13 Gene Analysis

We found 6 missense mutations (p.T339R, p.C438S, P.Q448E, p.P475S, p.P618A, and p.G909R) in this family (fig. 1C). Of these, p.T339R, p.Q448E, p. P475S, and p.P618S have been previously reported as single nucleotide polymorphisms (SNPs) in the Japanese population [14]. This patient had two mutations (p.C438S and p.G909R) that appear to be disease-causing mutations that have never been previously reported. We analyzed these two mutations using PolyPhen-2 to predict their effects on ADAMTS13. Both mutations were predicted to be 'probably damaging.' Thus, the patient was a compound heterozygote for two mutations in the *ADAMTS13* gene: p.C438S (c.1313G>C, exon 12) was inherited from her father and p.G909R (c.2725 G>A, exon 21) was inherited from her mother.

Clinical Course in Pregnancy

Although the patient had low levels of ADAMTS13 inhibitor, we diagnosed this patient with USS based on the results of the genetic analysis. Taking into account the risk of TTP, she chose elective abortion for her first pregnancy at 28 years of age. However, when she became pregnant again at the age of 30, she strongly hoped to have a child. After thorough discussions between the hematologists and obstetricians, we decided to continue the pregnancy with close monitoring of her condition and her fetus.

The patient's plasma ADAMTS13 activity was under 0.5% and ADAMTS13 inhibitor was negative at 8 weeks of gestation without FFP infusion. Starting at 9 weeks of gestation, 4 units of FFP were infused (480 ml / 92 kg body weight = 5.2 ml/kg). Between 11 and 17 weeks of gestation, the patient received 6 units of FFP (97 kg, 7.4 ml/kg) biweekly. In this period, ADAMTS13 activity was 3–4% of normal just before FFP infusion. At 17 weeks, she had fever with an upper respiratory infection. Her platelet count suddenly decreased to 141 × 10^9/l. Therefore, she received 6 units of FFP on the next day. Subsequently, 6 units of FFP were infused weekly, with plasma levels of ADAMTS13 activity measured before and after FFP infusion. After 32 weeks of gestation, the volume of FFP infusion increased to 8 units (103 kg, 9.3 ml/kg) per week. In addition to FFP infusion, she took low-dose aspirin (100 mg/day) between 9 and 34 weeks of gestation.

As shown in figure 2, this regimen maintained her platelet count over 200 × 10^9/l. Plasma levels of ADAMTS13 before FFP infusion were 3–5% of normal, and levels after FFP infusion were approximately 10%. The maximum level of ADAMTS13 inhibitor was 0.9 BU/ml at 20 weeks of gestation. Until 29 weeks of gestation, the levels of inhibitor were relatively high. However, after 30 weeks of gestation, inhibitor levels over 0.5 BU/ml were not observed except at 35 weeks (0.6 BU/ml). After delivery, ADAMTS13 inhibitor levels over 0.5 BU/ml were not detected. Moreover, levels of ADAMTS13 binding antibodies before pregnancy were over 30 units/ml (table 1). These levels gradually decreased as the pregnancy progressed, similar to levels of ADAMTS13 inhibitor. At 39 weeks of gestation, she gave birth to a healthy baby girl by cesarean section. She received 8 units of FFP on the day of surgery and 6 units on postoperative days 1, 3, and 5. Prophylactic FFP infusion was then stopped. After delivery, plasma levels of ADAMTS13 activity were maintained between 1.8 and 5.2%, and both ADAMTS13 inhibitor titers and IgG antibodies were almost undetectable on three different occasions without FFP infusion (table 1).

The birth weight of her baby was 3,474 g. External malformations were not found. The ADAMTS13 activity of the umbilical cord was 35.5%, and the level of inhibitor was 0.7 BU/ml. Pathological examination of the placenta revealed only mild infarcts in the periphery and at the insertion of the umbilical cord.

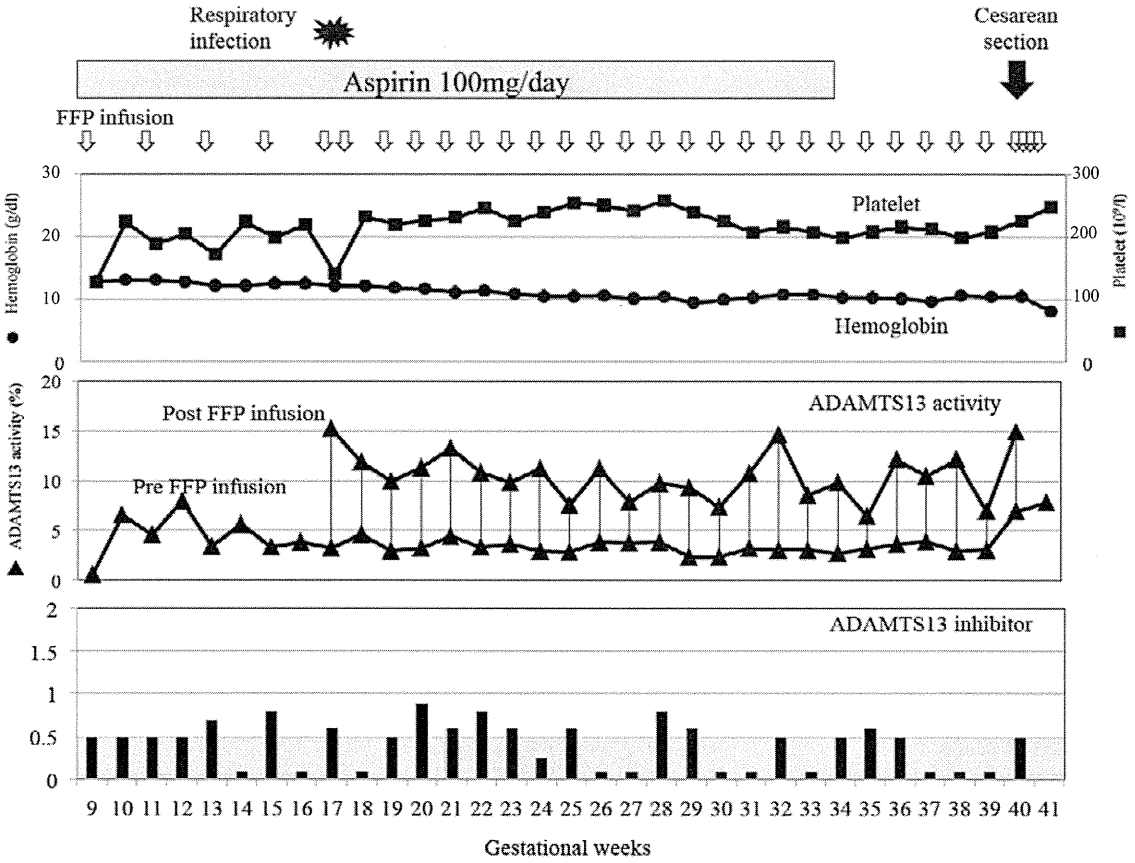


Fig. 2. Clinical course of USS-LL4 during pregnancy. FFP infusions were started at 5 ml/kg biweekly. At 17 weeks of gestation, she had an episode of respiratory infection. Her platelet count decreased from 200 to 140 × 10^9 /l. Subsequently, the interval between FFP infusions was shortened to 1 week. Plasma levels of ADAMTS13 activity before FFP infusion were 3–5% and those after FFP infusion were approximately 10%. Platelet counts were maintained over 200 × 10^9 /l. The patient took low-dose aspirin between 9 and 34 weeks of gestation. At 39 weeks, she gave birth to a healthy baby girl via cesarean section.

Discussion

We diagnosed patient described here with USS with low titer of ADAMTS13 antibodies based on the results of *ADAMTS13* gene analysis. When the patient first became pregnant at 28 years of age, we chose an elective termination due to the risk of developing TTP. However, in her second pregnancy at 30 years of age, we decided to continue the pregnancy with close monitoring of her condition and her fetus. Since plasma VWF levels increase with gestational age even in normal pregnancy [15], much more ADAMTS13 supplementation may be necessary in late gestation in USS patients. Thus, the therapeutic protocol for this patient involved dose escalation of FFP infusions, and the interval between infusions was gradually shortened with the progression of pregnancy, with frequent monitoring of ADAMTS13 activity levels.

In addition to FFP infusion, we used low-dose aspirin between 9 and 34 weeks of gestation. In our USS registry, one patient with USS (USS-L2) successfully gave birth to 4 babies (including twins) with taking low-dose aspirin during pregnancy [5]. Another patient successfully treated with FFP infusion and low-dose aspirin was reported by another group from our registry in Japan [6]. Antiplatelet agents such as aspirin, dipyridamole and ticlopidine, have been used in the acute treatment of acquired TTP. British treatment guidelines for TTP recommend low-dose aspirin during platelet recovery (platelet count >50 × 10^9/l) for patients with acquired TTP. Fetal loss in patients with USS is presumably caused by the disturbance of utero-placental circulation by platelet thrombi. Although there is only anecdotal evidence, low-dose aspirin in addition to FFP infusion may be effective in pregnant patients with USS.

In this patient, we identified the presence of both ADAMTS13 inhibitors and ADAMTS13 binding antibodies before pregnancy. Kentouche et al. [16] reported a similar patient in whom ADAMTS13 inhibitors were detected during pregnancy. However, binding ADAMTS13 antibodies were not detected by a commercially available assay (Technoclone) in stored samples in which ADAMTS13 inhibitors were detected. In contrast, both ADAMTS13 inhibitor and binding antibodies were detected in our patient (table 1).

We were concerned about increasing ADAMTS13 antibody titers with frequent antigen stimulation associated with FFP infusions. Thus, plasma levels of ADAMTS13 inhibitor and ADAMTS13 activity were analyzed each week before and after FFP infusion. However, increases in ADAMTS13 inhibitor were not observed as her pregnancy progressed. On the contrary, plasma levels of ADAMTS13 inhibitor decreased to marginal levels (<0.5–0.9 BU/ml) during pregnancy, and so far have not increased again after delivery. ADAMTS13 IgG antibodies also decreased to almost undetectable levels after delivery (table 1).

Regarding this interesting phenomenon, it is generally said that during pregnancy a mother has a natural intra-uterine allograft (fetus), which is regularly not rejected, indicating that immunological tolerance is up-regulated during this period [17]. In fact, it

has been reported that rheumatoid arthritis (RA) disease activity is often transiently lower during pregnancy [18]. However, unlike RA, in which disease activity flares up in 90% of patients within the first 3 months postpartum unless appropriate medications are given before delivery [19], both neutralizing and non-neutralizing antibodies against ADAMTS13 in our USS patient have not increased after delivery. Although we cannot fully explain this interesting phenomenon at present, it is possible that an immunological reset after delivery might be involved [16, 17]. So far, we have observed the patient for over 2 years after delivery, but much longer observation may shed a light on this difficult question.

Acknowledgments

This work was supported in part by research grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan, from the Ministry of Health, Labor, and Welfare of Japan and from Takeda Science Foundation.

Disclosure Statement

YF is a member of clinical advisory boards for Baxter BioScience.

References

1 Levy GG, Nichols WC, Lian EC, Foroud T, McClintick JN, McGee BM, Yang AY, Siemieniak DR, Stark KR, Gruppo R, Sarode R, Shurin SB, Chandrasekaran V, Stabler SP, Sabio H, Bouhassira EE, Upshaw JD Jr, Ginsburg D, Tsai HM: Mutations in a member of the *ADAMTS* gene family cause thrombotic thrombocytopenic purpura. Nature 2001;413:488–494.

2 Furlan M, Robles R, Galbusera M, Remuzzi G, Kyrle PA, Brenner B, Krause M, Scharrer I, Aumann V, Mittler U, Solenthaler M, Lämmle B: Von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. N Engl J Med 1998;339:1578–1584.

3 Fujimura Y, Matsumoto M, Isonishi A, Yagi H, Kokame K, Soejima K, Murata M, Miyata T: Natural history of Upshaw-Schulman syndrome based on *ADAMTS13* gene analysis in japan. J Thromb Haemost 2011;9(suppl 1):283–301.

4 Moatti-Cohen M, Garrec C, Wolf M, Boisseau P, Galicier L, Azoulay E, Stepanian A, Delmas Y, Rondeau E, Bezieau S, Coppo P, Veyradier A, French Reference Center for Thrombotic Microangiopathies: Unexpected frequency of Upshaw-Schulman syndrome in pregnancy-onset thrombotic thrombocytopenic purpura. Blood 2012;119:5888–5897.

5 Fujimura Y, Matsumoto M, Kokame K, Isonishi A, Soejima K, Akiyama N, Tomiyama J, Natori K, Kuranishi Y, Imamura Y, Inoue N, Higasa S, Seike M, Kozuka T, Hara M, Wada H, Murata M, Ikeda Y, Miyata T, George JN: Pregnancy-induced thrombocytopenia and TTP, and the risk of fetal death, in Upshaw-Schulman syndrome: a series of 15 pregnancies in 9 genotyped patients. Br J Haematol 2009;144:742–754.

6 Kato R, Shinohara A, Sato J: ADAMTS13 deficiency, an important cause of thrombocytopenia during pregnancy. Int J Obstet Anesth 2009;18:73–77.

7 Richter J, Strandberg K, Lindblom A, Strevens H, Karpman D, Wide-Swensson D: Successful management of a planned pregnancy in severe congenital thrombotic thrombocytopenic purpura: the Upshaw-Schulman syndrome. Transfus Med 2011;21:211–213.

8 Kinoshita S, Yoshioka A, Park YD, Ishizashi H, Konno M, Funato M, Matsui T, Titani K, Yagi H, Matsumoto M, Fujimura Y: Upshaw-Schulman syndrome revisited: a concept of congenital thrombotic thrombocytopenic purpura. Int J Hematol 2001;74:101–108.

9 Kato S, Matsumoto M, Matsuyama T, Isonishi A, Hiura H, Fujimura Y: Novel monoclonal antibody-based enzyme immunoassay for determining plasma levels of adamts13 activity. Transfusion 2006;46:1444–1452.

10 Kasper CK, Aledort L, Aronson D, Counts R, Edson JR, van Eys J, Fratantoni J, Green D, Hampton J, Hilgartner M, Levine P, Lazerson J, McMillan C, Penner J, Shapiro S, Shulman NR: Proceedings: a more uniform measurement of factor VIII inhibitors. Thromb Diath Haemorrh 1975;34:612.

11 Yagi H, Ito S, Kato S, Hiura H, Matsumoto M, Fujimura Y: Plasma levels of ADAMTS13 antigen determined with an enzyme immunoassay using a neutralizing monoclonal antibody parallel ADAMTS13 activity levels. Int J Hematol 2007;85:403–407.

12 Ishizashi H, Yagi H, Matsumoto M, Soejima K, Nakagaki T, Fujimura Y: Quantitative western blot analysis of plasma ADAMTS13 antigen in patients with Upshaw-Schulman syndrome. Thromb Res 2007;120:381–386.

13 Kokame K, Matsumoto M, Soejima K, Yagi H, Ishizashi H, Funato M, Tamai H, Konno M, Kamide K, Kawano Y, Miyata T, Fujimura Y: Mutations and common polymorphisms in *ADAMTS13* gene responsible for von Willebrand factor-cleaving protease activity. Proc Natl Acad Sci U S A 2002;99:11902–11907.

14 Kokame K, Kokubo Y, Miyata T: Polymorphisms and mutations of ADAMTS13 in the Japanese population and estimation of the number of patients with Upshaw-Schulman syndrome. J Thromb Haemost 2011;9:1654–1656.

15 Stirling Y, Woolf L, North WR, Seghatchian MJ, Meade TW: Haemostasis in normal pregnancy. Thromb Haemost 1984;52:176–182.

16 Kentouche K, Voigt A, Schleussner E, Schneppenheim R, Budde U, Beck JF, Stefanska-Windyga E, Windyga J: Pregnancy in Upshaw-Schulman syndrome. Hamostaseologie 2013;33:144–148.

17 Ostensen M, Villiger PM, Forger F: Interaction of pregnancy and autoimmune rheumatic disease. Autoimmun Rev 2012;11:A437–446.

18 Ostensen M, Villiger PM: The remission of rheumatoid arthritis during pregnancy. Semin Immunopathol 2007;29:185–191.

19 Amin S, Peterson EJ, Reed AM, Mueller DL: Pregnancy and rheumatoid arthritis: insights into the immunology of fetal tolerance and control of autoimmunity. Curr Rheumatol Rep 2011;13:449–455.

Clinical Case Reports

Open Access

CASE REPORT

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

Noritaka Yada[1], Masayuki Fujioka[2], Charles L. Bennett[3], Kazuya Inoki[4], Toyokazu Miki[1], Akihiko Watanabe[4], Toshiko Yoshida[5], Masaki Hayakawa[6], Masanori Matsumoto[6] & Yoshihiro Fujimura[6]

[1]Department of Emergency and Critical Care Medicine, Yodogawa Christian Hospital, Osaka, Japan
[2]Department of Emergency and Critical Care Medicine, Nara Medical University, Kashihara, Japan
[3]South Carolina Center of Economic Excellence for Medication Safety and Efficacy, the Southern Network on Adverse Reactions (SONAR), the South Carolina College of Pharmacy, the WJB Dorn Veterans Administration Hospital, Columbia, South Carolina
[4]Department of Gastroenterology, Yodogawa Christian Hospital, Osaka, Japan
[5]Department of Nephrology, Yodogawa Christian Hospital, Osaka, Japan
[6]Department of Blood Transfusion Medicine, Nara Medical University, Kashihara, Japan

Correspondence
Yoshihiro Fujimura, Department of Blood Transfusion Medicine, Nara Medical University, Shijyo-cho 840, Kashihara city, Nara, Japan. Tel: +81 744 22 3051;
Fax: +81 744 29 0771;
E-mail: yoshifuji325@naramed-u.ac.jp

Funding Information
This work was supported in part by research grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan, from the Ministry of Health, Labor, and Welfare of Japan and from Takeda Science Foundation.

Received: 4 July 2014; Accepted: 4 December 2014

doi: 10.1002/ccr3.196

Key Clinical Message

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

Keywords

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

Introduction

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

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

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

Case Report

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

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

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

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

Discussion

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

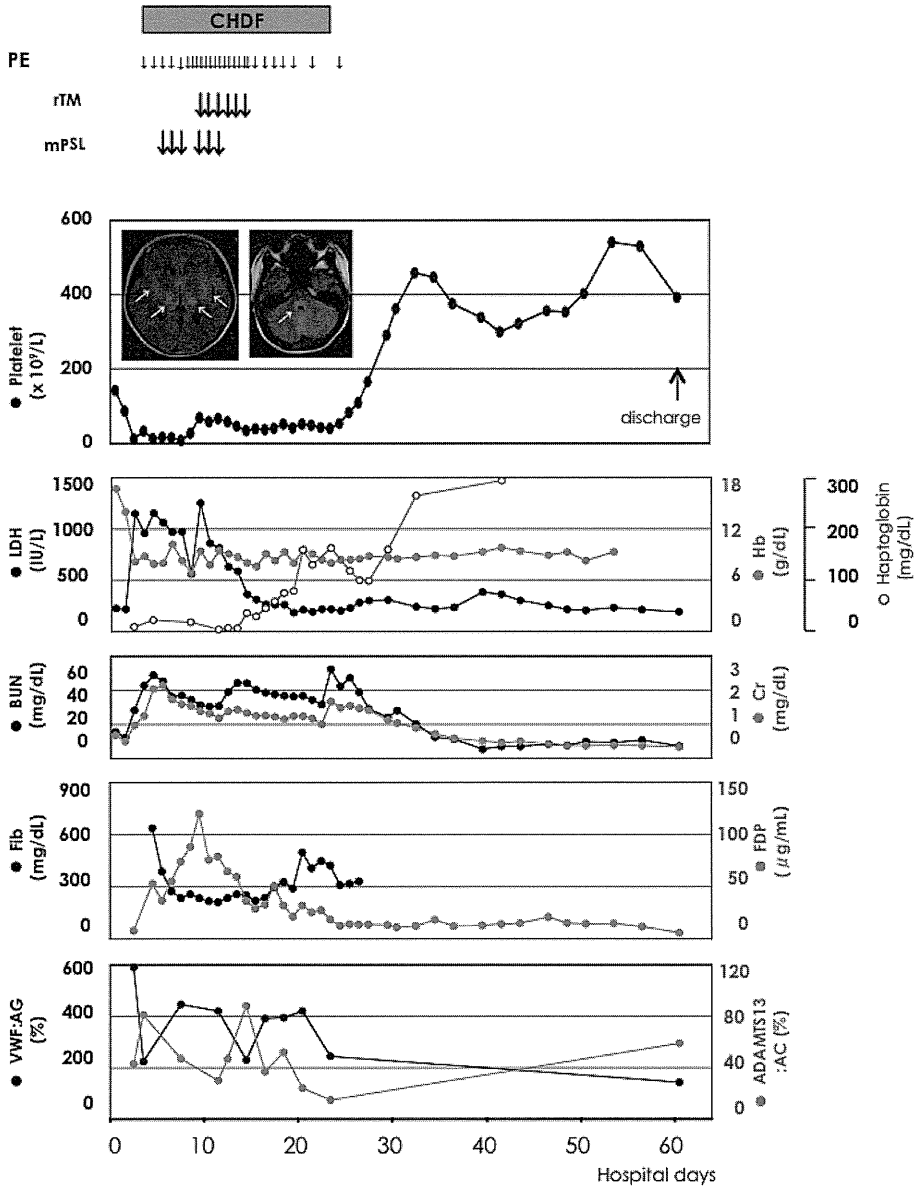


Figure 1. Clinical course in a 14-year-old girl with STEC-HUS complicated by acute encephalopathy after admission.

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

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

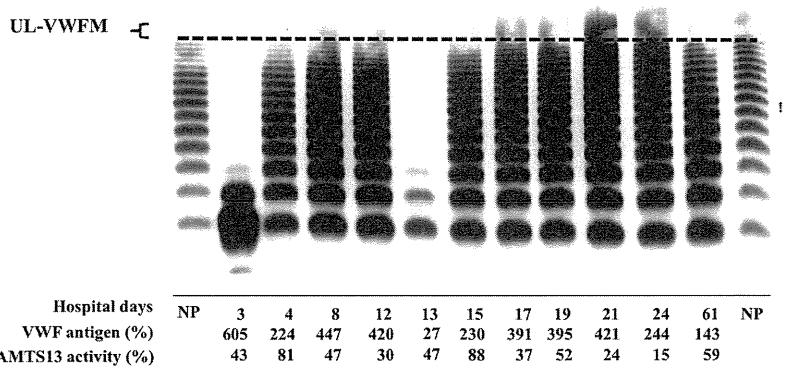


Figure 2. Change of VWF multimer patterns during the acute phase.

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

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

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

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

Acknowledgments

The authors wish to express our sincere gratitude to Prof. Hau C. Kwaan (Northwestern University Feinberg School of Medicine, Chicago) for his critical reading to this manuscript.