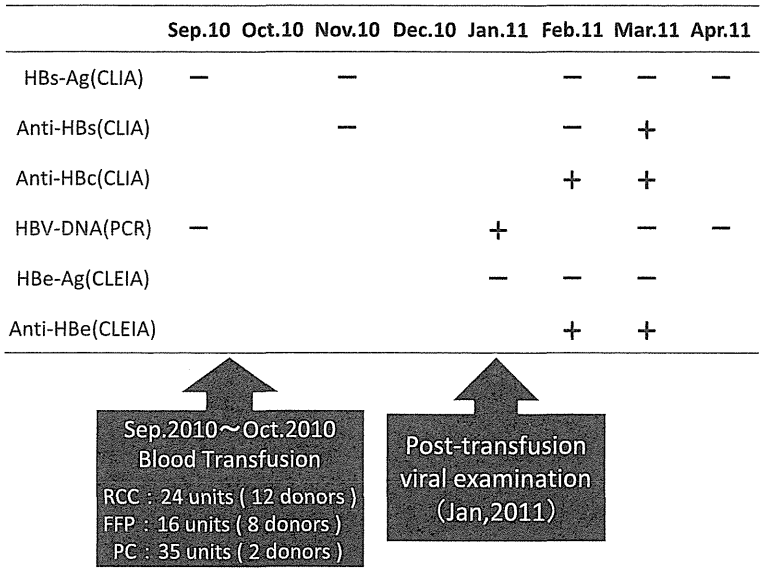

Fig. 1 Clinical course and changes in HBV marker
Between September 2010 and October 2010, the patient received 24 units of RCC derived from 12 donors and 16 units of FFP derived from 8 donors, and 35 units of PC derived from 2 donors.
The patient underwent post-transfusion viral examination in January 2011. Although, in September 2010, he showed negative for HBV markers, in January 2011 his HBV-DNA turned positive.
RCC: red cell concentrate, FFP: fresh frozen plasma, PC: platelet concentrate, CLIA: chemiluminescence immunoassay, CLEIA: Chemiluminescent Enzyme Immuno Assay, PCR: polymerase chain reaction, (+): positive, (−): negative

前のHBV感染は否定的であった. 2011年2月の再来院時にはHBc抗体とHBe抗体は陽性であったが, HBs抗原, HBe抗原およびHBV-NATは陰性化した. IgM-HBc抗体は測定していなかった. 臨床的に急性肝炎を疑う症状や肝機能検査値異常はなく, 投薬を含む肝炎関連の治療は行われていない.

2. 供血者検査

患者に用いられた血液製剤のドナー22名について遡及調査が行われた. 個別HBV-NATはすべて陰性であった. しかしながら, 本例の遡及調査中に, そのドナー22名のうちの1名が再度献血を行い, その血液を含む20本プールNATが陽性になり, さらに個別NATで検索した結果, 当該ドナーの血液がHBV陽性であることが判明した. 当該ドナーは1999年から年2回のペースで献血をしている複数回献血者であった. 2005年以降のデータでは常にHBc抗体は弱陽性(受身赤血球凝集反応にて抗体価16倍以下)であった. 本症例のHBV陽転原因となった血液製剤は当該ドナー由来のFFPであることが強く疑われた. このドナーに関しては過去の血液の遡及調査実施中とのことである.

3. 受血者と供血者のHBV-DNA相同性

日本赤十字社中央血液研究所にて患者の輸血後検体と当該供血者検体のHBV-DNAのα領域(PreS/S領域を含むP領域の前半部)の塩基配列をPCR direct sequence法により比較した結果, 99.2%の相同性を認めた. また, 両者のHBV-DNAはgenotype Cであり, 輸血によるHBV感染が強く疑われた. 塩基配列からsubtypeはadrと推察された.

考　察

供血者の感染症スクリーニングは血清学的検査に加え, 1999年10月から500本プールでNATが始まった. NATは2000年2月からは50本プール, 2004年8月からは20本プールになり, 2008年8月からは検出感度が向上した改良NATが実施されている. さらに2008年1月～7月にかけて血清学的検査は凝集法から化学発光免疫酵素測定法(CLEIA法)に変更された. その結果, 極めて安全な輸血製剤の確保が可能となっている. しかしながら, まだ年間10例前後の輸血HBV感染症例が報告されている[1)~4)]. その原因血の約半数がウインドウ期によるもので, 残りの半数は感染既往血(HBc抗体弱陽性)によるものである. これらの多くは個別NAT陽性によるものである. 本症例のようにHBc抗体弱陽性で個別NAT陰性の血液製剤が輸血され, HBV感染が確認されたのは第2報目である. 第1報は2006年に梶本らによって報告された[5)]. 輸血HBV感染の原

因と考えられたHBc抗体弱陽性・個別NAT陽性ドナー（occult HBV carrier）の遡及調査で，個別NAT陰性の同一ドナー由来血液の受血者3名に輸血HBV感染が確認された．これまでにHBV感染の初期であるHBs抗原とHBV-DNAが陰性を示すウインドウ期に献血された血液による急性B型肝炎発症の報告は散見されるが[6)~8)]，occult HBV carrierからの血液を輸血したことによる肝炎発症報告は少ない[9)10)]．Satakeらは日本における遡及調査で，ウインドウ期血液とoccult HBV carrierからの血液を輸血した際，感染性はウインドウ期が50%，occult HBV carrierが3%であり，感染性の強さに違いがあることを報告している[11)]．

日本赤十字血液センターはスクリーニングNATのプール本数を減らして検出感度を向上させてきた．また，検査技術の進歩によりNATの感度も向上しているが，完全にウイルスの感染を防ぐことはできないことが改めて示された．Occult HBV carrierの個別NATは検出感度以下となり，陰性になることはあるが，HBc抗体は弱いながらも陽性を示す．現在の日本赤十字社の輸血用血液製剤としての不適基準はCL4800システム（富士レビオ社）において，HBc抗体C.O.I≧12.0かつHBs抗体＜200mIU/m*l*となっている．北海道赤十字血液センターによると，現行基準での製剤不適率は0.2%であるが，感染既往血を排除するため検査基準をHBc抗体C.O.I≧1に引き下げた場合の不適率は3.1%と試算され，安定供給への影響は少なくないとしている．これは北海道がHBV感染率の高い地域であるからである．一方，石藤らの報告によると，東京管内のHBc抗体陽性率は北海道管内に比べて極めて低いので，基準を引き下げても影響は少ないと報告している[12)]．したがって，輸血用血液からの感染既往血排除については，安全性と安定供給の面から議論すべきことであるが，安全対策を強化する必要があろう．今後のHBc抗体検査基準変更やその他の安全対策に期待したい．

本症例は輸血から約4カ月後に輸血後感染症検査を行ったところでHBV-DNAが陽転していた．当院では最終輸血から2カ月以上経過した時点で，輸血後感染症検査対象患者を輸血部門システムから抽出し，病院情報システムに登録されている住所宛にダイレクトメール（DM）で「輸血後感染症検査のおすすめ」を送付している[13)]．また，輸血によるウイルス伝播の因果関係を証明する上で輸血前検体の検討は重要であるが，当院では交差適合試験後の患者血漿を約2m*l*分取し，約2年間凍結保存している．本症例では3本の輸血前患者血漿が凍結保存されていた．輸血前感染症検査は実施されていなかったが，輸血前保存検体の遡及検査で患者は輸血前にHBVに感染していなかったことを証明できた．その後，HBV-DNA相同性検査の結果，輸血によるHBV伝播が強く疑われた．当院では輸血前保存検体には交差適合試験用検体の残血漿を分取したものを凍結しているため，FFPやPCのみを輸血する患者については輸血前保存検体が確保できていないのが実情である．また，小児についても残血漿がほとんど残らないので，輸血前検体の確保はできていない．東海地区の医療機関における調査では[14)]，輸血前保存検体は，赤血球輸血に限れば84.9%の施設で保存されているが，FFP, PCを含むと輸血前の検体を保存している施設は40.3%であった．当院も含め，FFPやPCのみを輸血し，それが原因でウイルスに感染したか否かを輸血前検体で精査できない施設が多いと予想される．輸血が原因と考えられる感染例の調査や感染被害救済制度の適用のためには，すべての輸血症例に対し，輸血前検体を保存しておくシステムを構築しておくことが重要であろう．また，輸血前保存検体は輸血によるウイルス感染の証明に重要であるが，輸血後検査陽性例の輸血前の状況を知るためにも重要である[15)]．本症例を経験し，輸血前検体保存の重要性を改めて再認識し，当院でもFFP, PCのみを輸血する患者の輸血前検体保存について検討したいと考えている．

結　語

個別HBV-NAT陰性供血者からの輸血であったにも関わらず，HBV感染が強く疑われた症例を経験した．現時点ではoccult HBV carrierからの血液の輸血を確実に排除することは困難と考えられるが，今後何らかの対策が立てられることを期待する．輸血によるウイルス感染が疑われた場合，それを証明するためには輸血前検体保存が必須である．

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A HEPATITIS B VIRUS INFECTION BY BLOOD TRANSFUSION FROM AN OCCULT HEPATITIS B VIRUS CARRIER: A CASE STUDY

Daisuke Hanada[1], *Shuichi Kino*[1], *Yoshie Kawahara*[1], *Shiori Yamauchi*[1], *Yutaka Tomoda*[1], *Daisuke Kawata*[2], *Satoshi Fujita*[2], *Katsuya Morishita*[3], *Shinichiro Sato*[3] *and Hisami Ikeda*[3]

[1]Blood Transfusion and Cellular Therapy, Asahikawa Medical University Hospital

[2]Department of Emergency Medicine, Asahikawa Medical University

[3]Hokkaido Red Cross Blood Center

Abstract:

Although post-transfusion hepatitis B virus (HBV) infection has become rare, it has not been eliminated. We experienced a case of HBV infection due to blood derived from HBV nucleic acid amplification test negative donor.

A male patient with multiple injuries received in a traffic accident was admitted to our hospital. Between September 2010 and October 2010, he received 35 units of platelet concentrate derived from 2 donors, 24 units of red cell concentrate derived from 12 donors, and 16 units of fresh frozen plasma derived from 8 donors. At a post-transfusion viral test in January 2011, four months after the last transfusion, HBV-DNA became positive. A look-back study with stored blood samples of the 22 donors was carried out. The results of HBV-NAT of the 22 samples were all negative. The blood sample from one of the 22 donors turned out to be positive for HBV NAT screening, when be donated again. He had been weakly positive for anti-HBc and was regarded as an occult HBV carrier.

Analysis of HBV DNA sequences of the patient and donor showed 99.2% homology. This finding indicates that blood of occult HBV carriers who are negative for HBV-NAT causes transfusion-transmitted HBV infection.

Keywords:

transfusion-transmitted HBV infection, occult HBV carrier, individual NAT, post transfusion viral marker test

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Paradigm shift of childhood thrombotic thrombocytopenic purpura with severe ADAMTS13 deficiency

Hideo Yagi, Masanori Matsumoto, Yoshihiro Fujimura

Nara Medical University, Department of Blood Transfusion Medicine, Nara, 634-8522 Japan

Correspondence:
Yoshihiro Fujimura, Nara Medical University, Department of Blood Transfusion Medicine, 840 Shijo-Cho, Kashihara City, Nara, 634-8522 Japan.
malon@naramed-u.ac.jp

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Summary

Thrombotic thrombocytopenic purpura (TTP) is a life-threatening generalized disease with pathological conditions termed thrombotic microangiopathy (TMA). TTP is thought to predominantly affect adults and to rarely occur in children. Currently, TTP is defined by a severe deficiency in the activity of ADAMTS13, a metalloprotease that specifically cleaves unusually large von Willebrand factor multimers under high shear stress. Genetic mutations in and acquired autoantibodies to ADAMTS13 cause congenital TTP (termed Upshaw-Schulman syndrome [USS]) and acquired TTP, respectively. Because of very few overt clinical signs for TTP, USS is often misdiagnosed as chronic idiopathic thrombocytopenic purpura or overlooked during childhood. However, in women with USS, pregnancy can induce thrombocytopenia followed by the development of TTP. Furthermore, early childhood cases of acquired idiopathic TTP have not been characterized. From 1998 to 2008, our institution at Nara Medical University functioned as a TMA referral center in Japan and collected a large dataset on 919 TMA patients (Intern Med 2010;49:7–15). This registry contains 324 patients with a severe deficiency in ADAMTS13 activity, including 41 patients with USS and 283 patients with acquired TTP. Of note, the latter population contains 17 patients who were enrolled as children (≤ 15 years old), including 14 children with idiopathic TTP and three with connective tissue disease-associated TTP. Of the 14 patients with idiopathic TTP, five were very young children (under 2 years old). This study focused on these 58 patients (41 USS and 17 acquired TTP) who were diagnosed with a severe deficiency in ADAMTS13 activity during childhood, causing a paradigm shift in our concept of TTP.

Thrombotic microangiopathies (TMAs) are pathological conditions that are characterized by organ dysfunction due to platelet thrombi in the microvasculature, consumptive thrombocytopenia, and microangiopathic hemolytic anemia (MAHA). Two of the typical TMA phenotypes are life-threatening generalized diseases, termed thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) [1–4].

A disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13 (ADAMTS13) is a metalloprotease that specifically cleaves the Tyr1605–Met1606 bond in the von Willebrand factor (VWF)-A2 domain [5]. In the absence of ADAMTS13 activity (ADAMTS13:AC), unusually large VWF multimers (UL-VWFMs) are released from vascular endothelial cells (ECs) and improperly cleaved, causing them to accumulate in the circulation and induce the formation of platelet thrombi in the microvasculature under conditions of high shear stress. Currently, a severe deficiency in ADAMTS13:AC, which results either from genetic mutations in the *ADAMTS13* gene or acquired autoantibodies to ADAMTS13, is thought to be a specific feature of TTP but not HUS [6,7].

TTP was first described in 1924 by Moschcowitz [8], who documented a 16-year-old female who died of multiorgan failure after a clinical disease course of 1 week. An autopsy revealed hyaline membrane thrombi in the small arteries of multiple organs, except for the lung. In 1966, Amorosi and Ultmann [9] examined 16 new patients and reviewed 255 previously documented patients in order to establish a clinical 'pentad', consisting of MAHA, thrombocytopenia, renal failure, fluctuating neurological signs, and fever. Since then, TTP has been considered a life-threatening but rare disease that occurs mainly in adults and presents with predominant neurotropic clinical signs. Because of this classification, the estimated frequency of TTP was low (3.7 per million) [10] before the discovery of ADAMTS13.

On the other hand, in 1955 Gasser et al. [11] described five children who died of acute renal insufficiency, and their autopsies showed prominent necrosis of the renal cortex. This study established the clinical 'triad' for HUS, which consisted of MAHA, thrombocytopenia, and renal insufficiency. In addition, after it was determined that there was a close relationship between HUS and enterohemorrhagic *Escherichia coli* infection, particularly strain O157:H7 that produces a Shiga-like toxin, studies showed that HUS typically affects children with prominent nephrotropic clinical signs [12].

From 1998 to 2008, our institution at Nara Medical University has functioned as a TMA referral center in Japan and collected a large dataset of 919 patients who have TMA but not disseminated intravascular coagulation (DIC) [13]. This registry contains 324 patients with a severe ADAMTS13:AC deficiency (less than 3% of normal), including 41 patients with congenital TTP (Upshaw-Schulman syndrome [USS]) with variable clinical symptoms and 283 patients with acquired TTP. Notably, the latter population includes 17 patients who were diagnosed with TTP as children ($\leq$ 15 years old), including 14 with idiopathic TTP and three with connective tissue disease (CTD)-associated TTP. Surprisingly, the 14 patients with idiopathic TTP included five patients who were very young infants (under 2 years old), which significantly differed from the previous concept of TTP. Therefore, the aim of this study was to characterize these 58 patients (41 USS and 17 childhood TTP) in order to examine the paradigm shift in our understanding of TTP.

Glossary

ADAMTS13	a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13
ADAMTS13:AC	ADAMTS13 activity
ADAMTS13:INH	ADAMTS13 inhibitor
ai-TTP	acquired idiopathic TTP
BU	Bethesda unit
CR-TTP	chronic relapsing TTP
CTD	connective tissue disease
DIC	disseminated intravascular coagulation
EC	endothelial cell
FFP	fresh frozen plasma
HPS	hemophagocytic syndrome
HUS	hemolytic uremic syndrome
ITP	idiopathic thrombocytopenic purpura
MAHA	microangiopathic hemolytic anemia
PE	plasma exchange
PNH	paroxysmal nocturnal hemoglobinuria
SNP	single nucleotide polymorphism
TMA	thrombotic microangiopathy
TTP	thrombotic thrombocytopenic purpura
UL-VWFM	unusually large VWF multimer
USS	Upshaw-Schulman syndrome
VWF	von Willebrand factor
VWF-CP	VWF-cleaving protease
WPBs	Weibel-Palade bodies

Diagnostic criteria for thrombotic microangiopathy and thrombotic thrombocytopenic purpura

As previously described [13], patients were considered to have TMA if they met all of the following criteria:

- MAHA (hemoglobin [Hb] $\leq$ 12 g/dL), Coombs test negative, undetectable serum haptoglobin (< 10 mg/dL), more than two fragmented red blood cells (RBC) (schistocytes) in a microscopic field with a magnification of 100, and concurrent increased serum lactate dehydrogenase (LDH) above the institutional baseline;
- thrombocytopenia (platelet count $\leq 100 \times 10^9$/L);
- a variable degree of organ dysfunction (renal or neurological involvement) without DIC [14,15].

It is difficult to differentially diagnose HUS and TTP based on routine laboratory data. Therefore, as a rule, the plasma levels of ADAMTS13:AC were determined for all patients who were suspected to have TMA, and patients with a severe ADAMTS13:AC deficiency were classified as having TTP regardless of the clinical signs. This protocol was important because our registry included patients with congenital TTP or an ADAMTS13:AC deficiency (USS), which generally have fewer clinical signs, often isolated thrombocytopenia, than patients with acquired TTP.

Within the large dataset of 324 patients with a severe ADAMTS13:AC deficiency who were enrolled in our registry between 1998–2008 [10], 58 patients were diagnosed with a severe ADAMTS13:AC deficiency during childhood, of which 41 had congenital TTP (USS) and 17 were diagnosed with acquired TTP, including 14 with idiopathic TTP and three with CTD-associated TTP.

Assays for plasma ADMTS13:AC and ADAMTS13:INH

Until March 2005, ADAMTS13:AC was determined with a classic VWFM assay in the presence of 1.5 mol/L urea using purified plasma-derived VWF as a substrate according to the method described by Furlan et al. [16]. In our laboratory, the detection limit of this assay was 3% of the normal control [17].

In 2005, our laboratory developed a novel chromogenic ADAMTS13-act-ELISA using both an N- and C-terminal tagged recombinant VWF substrate (termed GST-VWF73-His). This assay was highly sensitive, and the detection limit was 0.5% of the normal control [18]. Since 2005, the classic VWFM assay was completely replaced with this novel chromogenic act-ELISA. Both assays show a high correlation between the plasma ADAMTS13:AC levels ($R^2 = 0.72$, $P < 0.01$) with similar means $\pm$ SD in healthy individuals ($102.4 \pm 23.0\%$ vs. $99.1 \pm 21.5\%$), as was shown previously [18]. Thus, the results obtained using the chromogenic act-ELISA were used in this study. In addition, we have categorized plasma ADAMTS13:AC levels of $< 3\%$, $3\sim < 25\%$, and $25\sim 50\%$ of the normal control as a severe, moderate, and mild deficiency, respectively.

Since 2005, ADAMTS13:INH has also been evaluated with the chromogenic act-ELISA by means of the Bethesda method [19]. Prior to this inhibition assay, the tested samples were heat-treated at 56 °C for 60 min to eliminate endogenous enzymatic activity. The ADAMTS13:INH assay consists of two steps. In the 1st step, the test or control plasma is heat-inactivated, mixed with an equal volume of intact normal pooled plasma, and incubated for 2 hours at 37 °C. After the incubation, the residual enzyme activity is measured. One Bethesda unit is defined as the amount of inhibitor that reduces the enzymatic activity by 50% of the control value, and values greater than 0.5 U/mL are considered significant.

Pathogenesis of thrombotic thrombocytopenic purpura

ADAMTS13-producing cells

ADAMTS13 is a metalloproteinase that consists of 1427 amino acids and a multi-domain structure, including a signal peptide, short propeptide, metalloproteinase domain, disintegrin-like domain, thrombospondin-1 (TSP1) domain, cysteine-rich domain, spacer domain, seven additional TSP1 repeats, and two CUB domains [20]. The *ADAMTS13* gene is located on chromosome 9q34, and initial northern blotting studies indicated that ADAMTS13 mRNA is exclusively expressed in the liver [20]. Subsequent immunological studies with *in situ* hybridization analyses indicated that ADAMTS13 is unambiguously produced in hepatic stellate cells (Itoh cells) [21]. However, ADAMTS13 was also identified in platelets [22], vascular ECs [23], and kidney podocytes [24]. Therefore, an outstanding and important question is which organ is most responsible for maintaining the plasma levels of ADAMTS13:AC. In this regard, we have two observations that suggest that the liver is the major ADAMTS13-producing organ. Childhood patients with advanced billiary cirrhosis due to bile duct atresia often showed pathological features of TMA with low plasma levels of ADAMTS13:AC (20–30%), but these clinical signs disappeared and plasma ADAMTS13:AC rapidly recovered to normal levels after a successful liver transplantation [25]. Adulthood patients with cirrhosis that was largely related to hepatitis C infection tended to have lower plasma ADAMTS13:AC levels that correlated with their clinical severity, and the lowest values were approximately 20–30% of the normal levels [26].

Cleavage of unusually large von Willebrand factor multimer

Although the mechanism by which TTP develops in the absence of ADAMTS13:AC has not been fully elucidated, accumulating evidence has provided a hypothesis as illustrated in *figure 1* [27]. In this proposed model, UL-VWFMs are produced exclusively in vascular ECs and stored in an intracellular organelle termed Weibel-Palade bodies (WPBs) and then released into the circulation upon stimulation. Under physiological conditions, epinephrine acts as an endogenous stimulus but other stimuli are largely unknown. In contrast, under non-physiological conditions, DDAVP (1-deamino-8-D-arginine vasopressin), hypoxia, and several cytokines such as interleukin (IL)-2, IL-6, IL-8, and tissue necrotizing factor (TNF)-α act as stimuli that up-regulate VWF release. Once ECs are stimulated, UL-VWFMs and P-selectin, both stored in WPBs, move to the membrane surface of ECs, where P-selectin anchors UL-VWFMs on the EC surface [28]. Under these circumstances, high shear stress generated in the microvasculature induces a change in the UL-VWFM molecule that alters its conformation from a

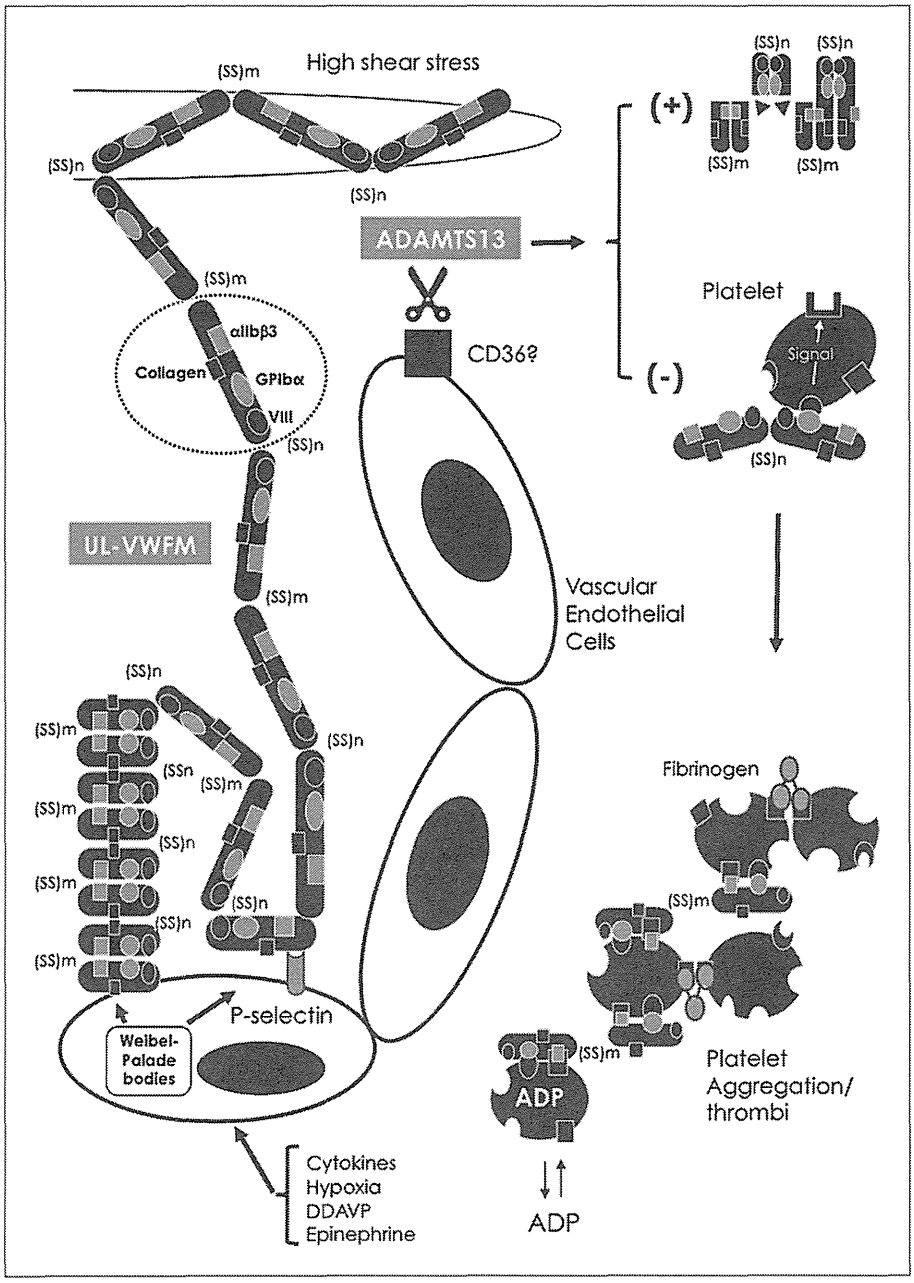

FIGURE 1

Proposed mechanism of platelet thrombi under high shear stress in the absence of ADAMTS13:AC

Unusually large von Willebrand factor multimers (UL-VWFMs) are produced in vascular endothelial cells (ECs) and stored in Weibel-Palade bodies (WPBs). UL-VWFMs are released from WPBs into the circulation upon stimulation by cytokines, hypoxia, DDAVP, and epinephrine. P-selectin that co-migrates from WPBs anchors UL-VWFMs on the vascular EC surface. Under these circumstances, high shear stress changes the molecular conformation of UL-VWFM from a globular to an extended form, allowing ADAMTS13 to access this molecule. In the absence of ADAMTS13:AC, UL-VWFMs are left uncleaved, allowing them to excessively interact with platelet glycoprotein (GP) Ibα and activate platelets via intraplatelet signaling, which results in the formation of platelet thrombi (dotted circle indicates a VWF subunit, which contains a set of binding domains with factor VIII, subendothelial collagen, platelet GPIbα, and integrin αIIbβ3).

globular to an extended form, allowing ADAMTS13 to cleave UL-VWFM. In this context, it has been postulated that multiple exocites within the disintegrin-like/TSP1/cysteine-rich/spacer (DTCS) domains of ADAMTS13 play an important role in interacting with the unfolded VWF-A2 domain [29]. Furthermore, although a direct link to TTP pathogenesis had not been shown, in 1994 Tandon et al. [30] reported that approximately 80% of patients with acquired TTP had autoantibodies to CD36. Recently, Davis et al. [31] showed that recombinant (r) ADAMTS13 specifically binds to rCD36 *in vitro*. Thus, it is possible that ADAMTS13 more efficiently cleaves newly released UL-VWFMs that exist as solid-phase enzymes

anchored to the vascular EC surface by binding to CD36 because CD36 is a receptor for TSP1, which is a repeated domain within the ADAMTS13 molecule.

In 2001, we clearly showed that pre-existing UL-VWFMs in the plasma of USS patients began to disappear within 1 hour and completely disappeared 24 hours after ADAMTS13 was replenished with infusions of fresh frozen plasma (FFP) as shown in *figure 2* [32]. Retrospectively, these results indicated that exogenous ADAMTS13 could efficiently cleave both UL-VWFMs that pre-existed in the circulation and the newly produced molecules at the EC surface. Related to this phenomenon, Zhang et al. [33] recently analyzed the crystal structure of the VWF-A2 domain and found that the ADAMTS13 cleavage site within this domain is not exposed to the outer surface of the molecule, indicating that the enzyme cannot readily access this site. More recently, Zanardelli et al. [34] proposed that the '2-site initial interaction mechanism between VWF and ADAMTS13', in which a binding site in the VWF C-terminal domains (D4CK) is constitutively exposed, allows this domain to interact with the ADAMTS13 C-terminal domains [TSP1(5-8)/CUB]. Under high shear stress, the '2-site initial interaction' may help expose this binding site within the VWF-A2 domain and favor the correct positioning of the ADAMTS13 spacer domain. Once the higher-affinity interaction between the spacer domain and the VWF-A2 domain is achieved, the metalloproteinase domain of ADAMTS13 can access and cleave the Tyr1605–Met1606 bond within the VWF-A2 domain.

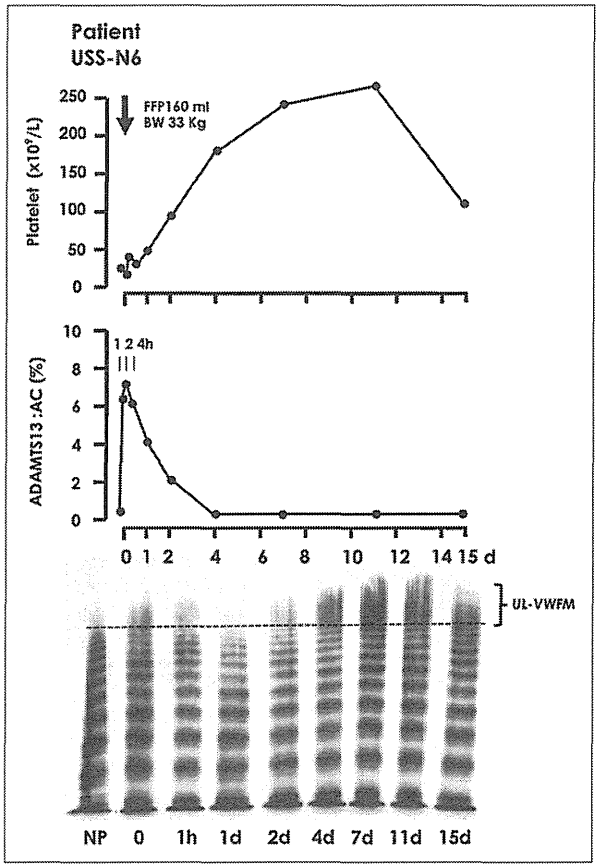

FIGURE 2

Effect of fresh frozen plasma (FFP) infusion on platelet counts, ADAMTS13:AC, and VWFM patterns in patient USS-N6

A total of 160 mL of FFP was transfused into female patient USS-N6 (BW 33 kg). As shown in the top panel, her platelet counts increased from 23 × 10^9/L before the FFP infusion to 251 × 10^9/L at 11 days after the infusion. The middle panel shows the plasma levels of ADAMTS13:AC that were re-examined by the chromogenic act-ELISA using deep-frozen plasma samples. Note that 4 days after the infusion, the plasma ADAMTS13:AC decreased to the pre-infusion level (< 0.5%). In the lower panel, the pre-existing UL-VWFM levels before the FFP infusion rapidly disappeared 24 hours after infusion, and 4 days later, UL-VWFMs re-appeared in the plasma. It should be noted that the platelet count began to decrease concomitantly with the re-appearance of UL-VWFMs (cited from [32] with a slight modification).

Anti-ADAMTS13 autoantibodies

Soejima et al. [35] were the first to report that the cysteine-rich and spacer domains of ADAMTS13 are a major binding site for ADAMTS13 autoantibodies in acquired TTP. Subsequently, Klaus et al. [36] showed that there are multiple antibody binding sites within the ADAMTS13 molecule. Now it is accepted that anti-ADAMTS13 neutralizing autoantibodies target epitopes within the spacer domain [37]. More recently, Pos et al. [38] identified three amino acids, Arg660, Tyr661, and Tyr665, within the spacer domain of ADAMTS13 that are critical for the binding of both the VWF-A2 domain and anti-ADAMTS13 autoantibodies.

Upshaw-Schulman syndrome (congenital TTP/deficiency in ADAMTS13:AC)

Background

The classic hallmarks of USS are repeated childhood episodes of chronic thrombocytopenia and MAHA that are reversed by infusing fresh frozen plasma (FFP). The most striking clinical feature is severe neonatal jaundice with a negative Coombs test that requires exchange blood transfusion therapy. Although USS is now defined as a congenital ADAMTS13:AC deficiency due to genetic mutations, there was a lengthy history that led to this conclusion, as has been described in detail in previous publications [39]. In fact, the term USS had almost been embedded in 1997, when the assay for VWF-cleaving protease (VWF-CP) activity (now ADAMTS13;AC) was established. This is because the pathogenic features that were initially postulated for the disease, such as a defect in 'platelet-stimulating factor', 'decreased plasma fibronectin

level', or 'lack of thrombopoietin', have been entirely excluded by subsequent investigations. Instead, the practical diagnostic term 'chronic relapsing TTP' (CR-TTP) has long been used. This term was coined by Moake et al. [40], who found that UL-VWFMs were present in the plasma of 4 CR-TTP patients during the remission phase, but disappeared during the acute phase. In 1997, Furlan et al. [41] showed that four CR-TTP patients, different from those of Moake et al. [40], lacked VWF-CP activity, but did not address ADAMTS13:INH. Retrospectively, however, each two CR-TTP patients, reported by Moake et al. [40] and Furlan et al. [41], were congenital TTP, and the remaining two each were acquired TTP. Under these circumstances, we re-visited the term USS [17], which included analyzing three Japanese patients with USS, and found that they uniformly had a severe deficiency in VWF-CP activity (determined by VWFM assay in the presence of 1.5 mol/L urea) in the absence of its inhibitors. The parents of these patients were asymptomatic with a moderately decreased activity (17–60% of normal), except for one carrier who had very low VWF-CP activity (5.6% of normal). Later, this carrier was shown to have a unique single nucleotide polymorphism (SNP), a P475S mutation in the *ADAMTS13* gene in one allele, which is very common in Japanese people (9.6% of normal individuals are heterozygous for the P475S mutation) [42]. However, Levy et al. [43] provided solid evidence that linked congenital TTP or USS and *ADAMTS13* gene mutations. Since this discovery, approximately 100 patients have been identified worldwide [44], but the precise incidence is completely unknown because USS is an extremely rare disease.

ADAMTS13 gene knock-out humans and mice

Although USS patients consistently lack ADAMTS13:AC, they do not always have acute symptoms, and symptoms often become evident only when the patients have infections or become pregnant. In both instances, vascular EC injuries might be involved, and these cases have been indirectly shown to have elevated plasma levels of cytokines or soluble thrombomodulin [45]. However, studies on *ADAMTS13* gene knock-out mice [46,47] showed that UL-VWFMs were detectable in the blood, although the mice did not have acute symptoms. Considering these results, investigators have assumed that an ADAMTS13:AC deficiency is prothrombotic but alone is insufficient to provoke acute symptoms. Therefore, second hits or triggers must exist. However, the lack of symptoms in knock-out mice sharply contrasts the clinical symptoms of USS. For example, USS patients, but not mice, were reported to have acute clinical aggravation soon after receiving infusions of DDAVP [48,49]. However, it is still controversial whether mice have a receptor to DDAVP. Furthermore, there are striking differences between humans and mice during pregnancy. In our studies, nine USS females had a history of pregnancy and all had thrombocytopenia during the 2nd–3rd trimesters. When this thrombocytopenia was not well managed, they developed clinical signs of TTP and the fetus died in many cases [50]. However, this disease course was not found in knock-out mice.

Natural history of 41 Upshaw-Schulman syndrome patients in Japan

USS is inherited in an autosomal recessive fashion, indicating that the female-to-male ratio in the patient population should be one-to-one. However, in our registry of 41 USS patients from 36 families (Table I), the female-to-male ratio was 25-to-16 with an apparent female predominance. Furthermore, all patients had a severe ADAMTS13:AC deficiency (under 3% of normal), except for one USS-GG2 patient (ADAMTS13:AC 2.4–3.4%).

Although severe neonatal jaundice is a typical sign of early-onset bouts of USS, our analysis indicates that such cases represent a relatively small number (16/41, 39%) of patients. Thirty-two patients (32/41, 78%) had repeated episodes of thrombocytopenia during childhood, but many USS patients were primarily misdiagnosed with idiopathic thrombocytopenic purpura (ITP) or Evans syndrome. Therefore, the age at which these patients were diagnosed with CR-TTP or USS was widely distributed from 1 month to 63 years. Sixteen patients (16/41, 39%) were diagnosed with TTP beyond childhood.

Of particular interest, pregnant women with USS inevitably have thrombocytopenia during the 2nd–3rd trimester when the plasma VWF levels rapidly increase with the appearance of UL-VWFM. *Figure 3* presents data for two female patients who were siblings in an USS-L family and were diagnosed based on their precise natural history around pregnancy followed by an examination of ADAMTS13:AC and ADAMTS13:INH [50]. Furthermore, an *ADAMTS13* gene analysis gave a solid diagnosis of USS. As illustrated in these two cases, USS is thought to have two clinical phenotypes, the early-onset and late-onset types. However, generally we cannot find any clear differences in the plasma ADAMTS13:AC levels between these two phenotypes, even when examined by the sensitive act-ELISA.

Regarding severe renal complications in USS patients, we know that two patients thus far have received hemodialysis for chronic renal insufficiency. One patient, USS-C3 (male, born in 1972), was diagnosed with USS at 8 years of age, and then given prophylactic FFP infusions that were continued for the rest of his life. However, his renal function deteriorated yearly and he began to receive hemodialysis when he was 24 years old. During the clinical course of his disease, he experienced chronic heart failure and died of a sudden cardiac attack at 38 years of age. The other patient, USS-JJ3 (male, born in 1980), was diagnosed with USS at 16 years of age, after which he received prophylactic FFP infusions. However, his renal function deteriorated and he has been receiving hemodialysis since he was 26 years old.

TABLE I

Registration of 41 Japanese patients with Upshaw-Schulman syndrome (USS)

No	Patient	Year of birth	Sex	Exchange blood transfusion during newborn period	Thrombocytopenia during childhood	ADAMTS13:AC (%)	Disease-causing ADAMTS13 gene mutations		Age of TTP diagnosis	Prophylactic FFP infusion		Remarks	Ref.
											From when		
1	A4	1999	M	+	+	< 0.5	C-Hetero	p.R268P/ p.C508Y	4 m	+	4 m		[53]
2	B3	1986	F	+	+	< 0.5	Homo	p.Q449X	2 m	+	11 m		[53]
3	C3	1972	M	–	+	< 0.5	Homo	c.414+1G>A	8 y	+	8 y	Dead (chronic heart failure at the age of 36)	[54]
4	D4	1978	F	+	+	< 0.5	C-Hetero	c.414+1G>A/ p.I673F	4 y	+	4 y		[54]
5	E4	1985	M	+	+	< 0.5	C-Hetero	p.I673F/ p.C908Y	5 y	–	–		[54]
6	F3	1993	M	+	+	0.6	C-Hetero	p.R193W/ p.1244+2 T>G	2.5 y	–	–		[54]
7	G3	1987	F	+	+	< 0.5	C-Hetero	c.686+1G>A/ p.R1123C	14 y	–	–		[54]
8	H3	1951	M	–	–	0.6	C-Hetero	p.A250V/ c.330+1G>A	51 y	+	50 y	Dead (renal failure at the age of 51)	[51]
9	I4	1972	M	–	+	< 0.5	C-Hetero	p.H234Q/ p.R1206X	2 y	+	2 y		[55]
10	J3	1977	F	–	+	< 0.5–0.8	C-Hetero	p.R312C/ c.3198del CT	3 y	+	22 y		[56]
11	J4	1979	M	–	+	< 0.5	C-Hetero	p.R312C/ c.3198del CT	5 y	–	–		[56]
12	K3	1976	F	–	+	< 0.5–0.7	C-Hetero	p.Y304C/ p.G525D	27 y	+	27 y		[50]
13	K4	1978	F	+	+	< 0.5	C-Hetero	p.Y304C/ p.G525D	25 y	+	25 y		[50]
14	L2	1967	F	–	–	< 0.5	C-Hetero	p.R125VfsX6/ p.Q1302X	25 y	–	–		[50]
15	L3	1972	F	–	+	< 0.5	C-Hetero	p.R125VfsX6/ p.Q1302X	25 y	–	–		[50]

TABLE I (*Continued*)

No	Patient	Year of birth	Sex	Exchange blood transfusion during newborn period	Thrombocytopenia during childhood	ADAMTS13:AC (%)	Disease-causing ADAMTS13 gene mutations		Age of TTP diagnosis	Prophylactic FFP infusion		Remarks	Ref.
											From when		
16	M3	1969	F	–	–	< 0.5	C-Hetero	p.R193W/ p.R349C	33 y	–	–		[50]
17	M4	1971	F	–	–	< 0.5	C-Hetero	p.R193W/ p.R349C	30 y	–	–		[50]
18	N6	1986	F	+	+	< 0.5	C-Hetero	p.H234R/ c.3220delTACC	4 y	+	4 y		[17]
19	O4	1958	F	–	–	< 0.5	C-Hetero	p.I178T/ p.Q929X	26 y	+	26 y		[50]
20	P3	1971	M	-	+	< 0.5	C-Hetero	p.C908Y/ p.C322G, p.T323R, p.F324L	3 y	+	21 y		[42]
21	Q1	1983	M	+	+	< 0.5–0.7	C-Hetero	p.G227R/ p.C908Y	6 y	+	11 y		[56]
22	Q2	1988	M	+	+	< 0.5	C-Hetero	p.G227R/ p.C908Y	2 y	+	7 y		[56]
23	R5	1982	F	–	+	< 0.5	C-Hetero	p.R193W/ p.A606P	23 y	+	23 y		[50]
24	S3	1982	F	–	+	0.9	Not determined		4 y	+	*		
25	T4	1981	F	+	+	< 0.5	Homo	c.3220delTACC	1 m	+	*		[56]
26	U3	1990	F	+	+	< 0.5	Homo	c.2259delA	4 m	+	*		[56]
27	V3	1983	F	+	+	< 0.5	C-Hetero	p.W1081X/ p.R193W	6 y	+	6 y		[56]
28	W4	1990	F	–	+	< 0.5	C-Hetero	p.G550R/Not determined	15 y	+	15 y		[56]
29	X5	1963	F	–	–	< 0.5	Not determined		40 y	–	–		
30	Y3	1960	F	–	+	< 0.5	C-Hetero	p.G385E/ p.R1206X	45 y	+	45 y		[56]
31	Z3	1971	F	–	+	< 0.5	Homo	p.R193W	25 y	–	–		[50]
32	AA3	1987	F	–	–	< 0.5	Not determined		19 y	–	–		
33	BB3	1947	M	–	–	< 0.5	Homo	p.R193W	55 y	–	–		[56]
34	CC5	2004	M	+	+	< 0.5	C-Hetero	p.Q723K/ p.R398C	2 y	+	2 y		[56]

TABLE I (*Continued*)

No	Patient	Year of birth	Sex	Exchange blood transfusion during newborn period	Thrombocytopenia during childhood	ADAMTS13:AC (%)	Disease-causing ADAMTS13 gene mutations		Age of TTP diagnosis	Prophylactic FFP infusion		Remarks	Ref.
										From when			
35	DD5	2007	F	–	+	< 0.5	C-Hetero	p.R268P/ p.Y304C	1 m	–	–		[56]
36	EE4	2003	M	+	+	< 0.5	Homo	c.2259delA	4 y	–	–		[56]
37	FF3	1991	F	–	+	< 0.5	Homo	p.Q449X	6 y	+	6 y		[56]
38	GG2	1931	M	–	–	2.4–3.4	Homo	p.C1024R	63 y	+	63 y	Dead (stroke at the age of 79)	[56]
39	HH4	2003	F	+	+	< 0.5	C-Hetero	p.Q449X/ c.4119delG	1 y	–	–		[56]
40	II3	1977	F	+	+	< 0.5	Not determined		9 m	+	10 y		
41	JJ3	1980	M	–	+	< 0.5	C-Hetero	c.1885delT/ p.C908Y	12 y	+	25 y	Hemodialysis	[56]

C-Hetero: compound heterozygotes; Homo: homozygotes.

There have been two fatal USS cases, one is the above-mentioned USS-C3 and the other is patient USS-I4 (male, born in 1972), whose natural history was previously described in detail [51]. Briefly, patient USS-I4 was diagnosed with late-onset USS when he was 50 years old. The next year he received a cholecystectomy and then experienced a bout of TTP, which led to renal insufficiency. He received extensive treatment, including PE and hemodialysis but did not improve, and he died of renal insufficiency at the age of 51 years.

ADAMTS13 gene analysis

The parents of USS patients are usually asymptomatic carriers, and a major population of patients from unrelated parents is a compound heterozygote, while a minor population of patients from related parents is a homozygote [42,43,51–56].

We performed *ADAMTS13* gene analyses in 38 out of 41 USS patients and disease-causing mutations were identified in 37 patients: nine with homozygous and 28 with compound heterozygous *ADAMTS13* gene mutations. Furthermore, five of these 37 patients were siblings. Therefore, within 64 [2 × (37 – 5)] allelic numbers (*n*) for *ADAMTS13* gene mutations, the three most frequently found mutations were in the following order: p.R193W (*n* = 8), p.Q449X (*n* = 5), and p.C908Y (*n* = 4). All these mutations were unique to Japanese individuals, perhaps to East-Asians, and were totally different from Europeans and white and black Americans. In addition, to date, we have not found an apparent association between specific *ADAMTS13* mutations and clinical phenotypes. However, Camilleri et al. [57] reported that some single nucleotide polymorphisms in the *ADAMTS13* gene could modulate ADAMTS13:AC and its secretion, indicating that further investigations are required.

Patient USS-GG2 (male, born in 1931) suddenly developed a bout of TTP when he was 63 years old. After this incident, he had repeated TTP bouts and required prophylactic FFP infusions under a clinical diagnosis of CR-TTP. Even under these circumstances, he developed a cerebellar infarction at 76 years of age. During the infusion intervals, ADAMTS13:AC was often measured and determined to be 2.4–3.4% of the normal levels but ADAMTS13:INH was not detected. Most recently, the patient was diagnosed with USS with the homozygous missense mutation C1024R based on an *ADAMTS13* gene analysis (unpublished).

Treatment

Except for exchange blood transfusions to treat jaundice in newborns, USS patients usually respond well to small FFP infusions. Therefore, the question arises; what is the best marker for deciding this indication? As suggested above, mild thrombocytopenia seems to occasionally occur in USS patients during childhood, but this condition might be overlooked

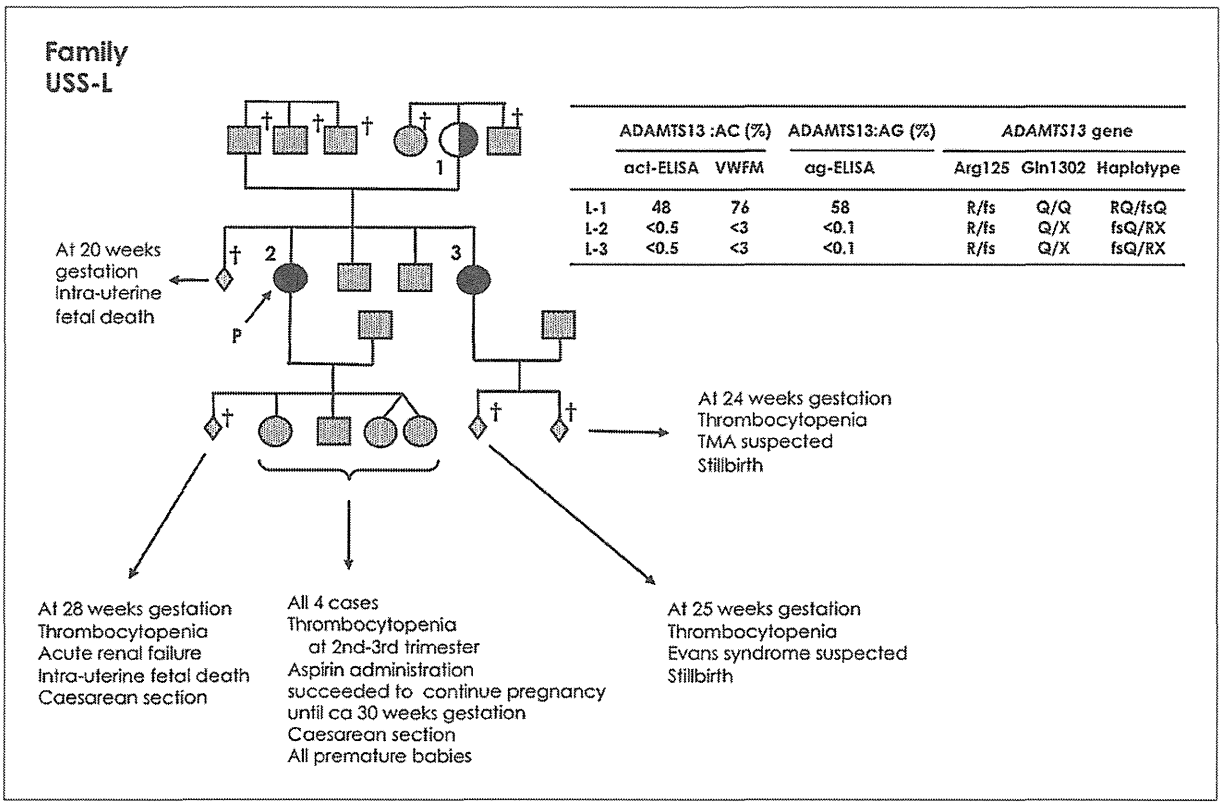

FIGURE 3

Family pedigree and ADAMTS13 analyses in family USS-L

The propositus is L2, and L3 is her younger sister. Both siblings had an abortion along with thrombocytopenia of an unknown etiology. When propositus L2 became pregnant the second time, she had mild thrombocytopenia and her physician recommended low-dose aspirin, which enabled her to maintain the pregnancy until 30 weeks of gestation. However, it was uncertain why and how aspirin worked in this occasion. She delivered a live but premature baby by caesarean section. Then, she successfully bore three more children with the same treatment. All of the babies were premature and alive. During childhood, L2 had no episodes of thrombocytopenia, but L3 was diagnosed with ITP at 3 years of age. At 25 years of age, propositus L2 was diagnosed with USS based on an analysis of ADAMTS13:AC and ADAMTS13:INH. In addition, an *ADAMTS13* gene analysis provided solid evidence that the two siblings are compound heterozygotes for *ADAMTS13* gene mutations (p.R125fsx6/p.Q1302X). Squares and circles indicate males and females, respectively, and an arrow with P indicates the proposita. Closed circles and closed squares represent USS patients. The half-closed circles and squares represent asymptomatic carriers. The cross indicates deceased (cited from [50] with a slight modification).

because of the paucity of clinical signs. Thus, mild thrombocytopenia alone may not be a good marker. However, in clinical practice, some USS patients receive prophylactic FFP infusions (5–10 mL/kg BW) every 2–3 weeks because the half-life of ADAMTS13:AC in the plasma is thought to be 2–3 days, while other patients receive FFP infusions only when acute TTP bouts develop. In our registry, 26 of 41 (63%) USS patients received prophylactic FFP infusions. Currently, USS patients receive FFP infusions based on the physician's observations and the frequency of TTP bouts. However, the efficacy of prophylactic FFP infusions needs to be more precisely evaluated over a long observation period because our two patients who developed renal insufficiency had been receiving FFP infusions since they were clinically diagnosed with CR-TTP.

One serious adverse effect of repeated plasma infusions is that nine out of 41 (22%) USS patients were infected with hepatitis C virus. In this regard, virus-free rADAMTS13 preparations would be a promising product for USS patients.

Acquired thrombotic thrombocytopenic purpura/ADAMTS13:AC deficiency

Figure 4 shows age and gender distribution of acquired TTP. Of 195 patients with acquired TTP and a severe ADAMTS13:AC deficiency due to ADAMTS13:INH, 17 (6%) were childhood patients, including 14 with acquired idiopathic TTP (ai-TTP)

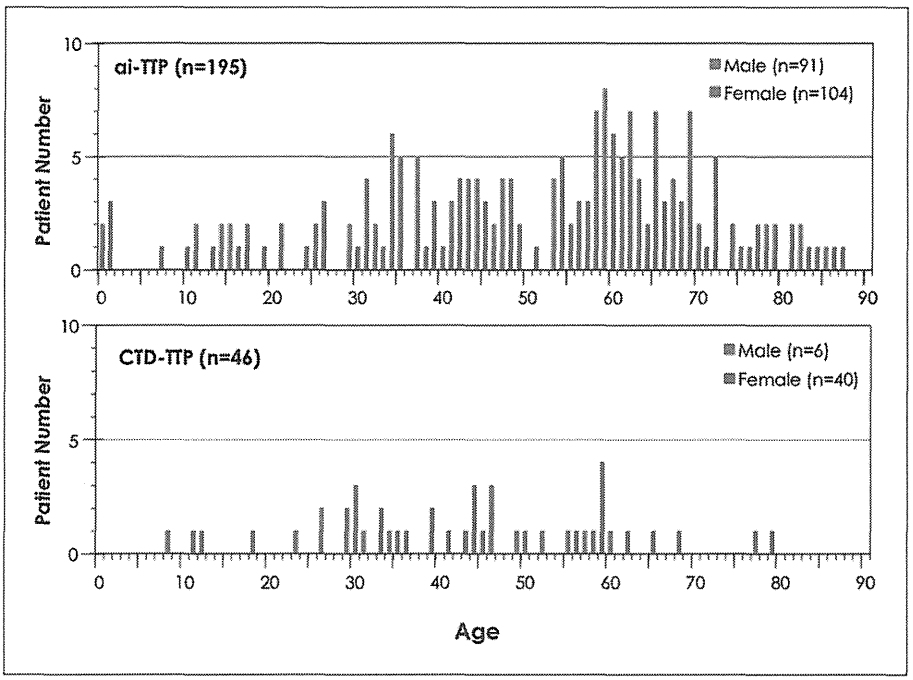

FIGURE 4

Age and gender distribution of patients with acquired idiopathic (ai)-thrombotic thrombocytopenic purpura (TTP) and connective tissue disease (CTD)-associated TTP

The upper panel shows the age and gender distribution of 195 ai-TTP patients who were registered at Nara Medical University during 1998–2008 and were determined to have a severe ADAMTS13:AC deficiency and be positive for ADAMTS13:INH. The largest population peak is found at approximately 60 years old, and we identified 14 patients (14/195, 7.2%) aged less than 15 years. Of note, five were very young children below 2 years of age.

The lower panel shows the age and gender distribution of 46 CTD-TTP patients who were registered at our institution during the same time period and were determined to have a severe ADAMTS13:AC deficiency and be positive for ADAMTS13:INH. This CTD-TTP patient population is widely distributed across ages, but is more common in patients between 30–60 years old. There is an apparent female predominance (41/46, 89%) in CTD-TTP patients in this registry, and three childhood patients were identified.

and three with connective tissue disease (CTD)-associated TTP. Of note, the former group included five patients who were less than 2 years of age and were initially misdiagnosed with other thrombocytopenic disorders, such as ITP, HUS, and hemophagocytic syndrome (HPS). It is important to examine and compare the detailed clinical features of these TTP patients to adulthood patients in order for physicians to ascertain that TTP is not a rare childhood disease. Therefore, we herein describe the clinico-laboratory features of these five young infants with ai-TTP and three childhood patients with CTD-associated TTP.

Idiopathic (ai-) thrombotic thrombocytopenic purpura

Table II summarizes the features of the 14 childhood patients with ai-TTP, including five infantile patients. Case 1 was previously reported [58] and case 5 was more recently described [59]. Interestingly, in contrast to the adulthood ai-TTP patients with a severe ADAMTS13:AC deficiency, the childhood patients had a slightly male predominance (female:-male = 5:9). Five patients (5/14, 33%) had apparent prodromal illnesses, such as an upper respiratory tract infection (n = 3), Rotavirus infection (n = 1), or urinary tract infection (n = 1). Ten patients (10/14, 67%) had neurological findings, including headache (n = 5), altered mental status (n = 4), hemiparesis (n = 1), seizures (n = 1), and vision disturbance (n = 1). These patients exclusively presented with renal involvement (11/14, 73%) and fever (13/14, 93%). All the patients had hemolytic anemia (Hb, 4.5–11.3 g/dL) and thrombocytopenia (platelet, 7–38 $\times$ 10^9/L), but their serum creatinine levels (Cr, 0.19–1.0 mg/dL) remained within the normal range. Most of the childhood patients had five clinical signs that are characteristic of classic TTP ('pentad'), but six patients, including five young infants aged below 2 years, were initially misdiagnosed with other thrombocytopenic disorders, such as ITP (n = 2), HUS (n = 2), HPS (n = 1), and paroxysmal

TABLE II

Clinical features in childhood patients with acquired TTP with severe ADAMTS13 deficiency

Case	Age	Sex	Prodromal illness	Initial diagnosis	Clinical findings on admission					
					Neurological symptom	Renal involvement	Cr (mg/dL)	Fever	Hb (g/dL)	Platelet (×109/L)
1	9 m	F	Rotavirus	HUS	Altered mental status	Yes	0.3	Yes	4.5	2
2	19 m	F	URI	ITP	No	Yes	0.19	Yes	11.3	38
3	19 m	M	URI	HUS	Altered mental status	Yes	1	Yes	5.4	9
4	12 m	M	No	HPS	Hemiparesis	Yes	0.31	Yes	8.5	38
5	8 m	M	No	ITP	No	No	0.19	Yes	8.7	25
6	7 y	M	No	TTP	Altered mental status	No	0.32	Yes	5.4	7
7	10 y	M	UTI	TTP	No	Yes	0.4	No	10.5	19
8	11 y	M	No	TTP	Headache	Yes	0.7	Yes	8.8	3
9	11 y	F	URI	TTP	No	Yes	0.6	Yes	5.5	6
10	13 y	F	No	TTP	Headache	No	0.58	Yes	5.7	12
11	14 y	F	No	TTP	Altered mental status, convulsion	No	0.4	Yes	7.8	6
12	15 y	M	No	PNH	Headache	Yes	1	Yes	10.5	17
13	15 y	M	No	TTP	Headache	Yes	0.91	Yes	8.1	11
14	14 y	M	No	TTP	Headache, visual disturbance	Yes	1.05	Yes	7	28
15	8 y	F	SLE	DIC	Altered mental status, headache	Yes	0.44	Yes	8.8	38
16	11 y	F	MCTD	TTP	Headache	Yes	0.6	Yes	11.9	43
17	12 y	F	SLE	TTP	No	No	0.61	Yes	5.6	1

Case	ADAMTS13: AC (%)	ADAMTS13: INH (BU/ml)	Treatments				Outcome		Clinical course remarks	Ref.
			PE (times)	FFP infusions	Immunosupressive agents	Platelet transfusion	Relapse	Prognosis		
1	< 0.5	> 100	19	No	SP	Yes	No	Alive	Cerebral infarction	[58]
2	< 0.5	4.3	3	Yes	SP, PSL	Yes	Yes	Dead		
3	< 0.5	2.3	3	Yes	PSL	Yes	No	Alive		
4	< 0.5	1.7	2	No	PSL	Yes	No	Alive	Cerebral infarction	
5	< 0.5	4.8	6	No	SP, PSL	Yes	No	Alive		[59]
6	< 0.5	2.1	3	No	Rituximab	No	No	Alive		
7	< 0.5	0.5	No	No	SP, PSL, MZR	Yes	No	Alive		
8	< 0.5	1.3	No	Yes	SP	No	No	Alive		
9	< 0.5	5.6	5	No	PSL	Yes	No	Alive		
10	< 0.5	2	9	No	SP, PSL	No	No	Alive		

TABLE II (*Continued*)

Case	ADAMTS13: AC (%)	ADAMTS13: INH (BU/ml)	Treatments				Outcome		Clinical course remarks	Ref.
			PE (times)	FFP infusions	Immunosupressive agents	Platelet transfusion	Relapse	Prognosis		
11	< 0.5	3.2	17	No	SP, PSL, VCR	No	No	Alive		
12	< 0.5	34	39	No	PSL, VCR, CSA	No	Yes	Alive		
13	< 0.5	2.3	3	No	SP, PSL, AZT	No	No	Alive		
14	< 0.5	6.8	30	Yes	PSL	No	No	Alive		
15	< 0.5	1.2	5	Yes	SP, PSL, CY	No	No	Alive		
16	< 0.5	1.8	3	No	SP, PSL	No	No	Alive		
17	< 0.5	0.7	9	Yes	SP, PSL	No	No	Alive		

Cr: creatinine; Hb: hemoglobin; ADAMTS13:AC: ADAMTS13 activity; ADAMTS13:INH: ADAMTS13 inhibitor; PE: plasma exchange; FFP: fresh frozen plasma; URI: upper respiratory infection; UTI: urinary tract infection; SLE: systemic lupus erythematosus; MCTD: mixed connective tissue disease; TTP: thombotic thrombocytopenic purpura; ITP: idiopathic thrombocytopenic purpura; HUS: hemolytic uremic syndrome; HPS: hemophagocytic syndrome; PNH: paroxysmal noctural hemoglobinuria; SP: steroid pulse; PSL: predonosolone; MZR: *mizoribine*; VCR: *vincristine*; CSA: *cyclosporine A*; AZT: *azathioprine*; CY: *cyclophosphamide*.

nocturnal hemoglobinuria (PNH, $n = 1$). After analyzing ADAMTS13, they were all correctly diagnosed with ai-TTP.

Of these 14 childhood patients with ai-TTP, 13 received plasma exchange (PE, 2–39 times, median 5 times), including four patients who subsequently received a FFP infusion. They also received immunosupressive therapy, including steroid pulse ($n = 7$), predonisolone ($n = 12$), vincristine ($n = 2$), cyclosporin ($n = 1$), azathioprine ($n = 1$), mizoribine ($n = 1$), and rituximab ($n = 1$). As an adjunctive therapy, the patients were given intravenous immune globulin ($n = 3$) or an antiplatelet agent ($n = 2$). Of note, seven patients received platelet transfusions before or after they were diagnosed with acquired TTP. In five of the seven patients who received platelet transfusions, there were no apparent serious complications. However, case 1 developed general convulsions soon after the platelet transfusion, and case 2 died from bleeding without appreciable hemostatic effects from the platelet transfusion. As consequence, 13 out of 14 childhood patients with ai-TTP achieved one clinical remission, but two patients relapsed, including one who died. We think that clinicians should be aware of the existence of ai-TTP during very early childhood, and herein we present a short summary for each of these five infants with ai-TTP.

Case 1

In March 2000, a 9-month-old girl presented with a fever. She subsequently showed loss of appetite, a drop in physical activity, a pale complexion, and vomiting followed by diarrhea related to a Rotavirus infection. On the following day, these symptoms continued and generalized petechiae appeared. She was taken to a family doctor who determined that she had severe anemia and thrombocytopenia. As a result, she was admitted to a nearby hospital for treatment. Upon admission, she was drowsy and her laboratory findings showed severe anemia (Hb, 4.5 g/dL), thrombocytopenia (platelet, 2.0×10^9/L), hyperbilirubinemia (total bilirubin, 2.6 mg/dL), and an elevated LDH level (2,925 IU/L). Both direct and indirect Coombs' tests were negative, and fragmented RBCs were detected in the blood film. The hemostatic tests showed the following: prothrombin time (PT, 14.0 sec), activated PTT (35.9 sec), fibrinogen (268 mg/dL), thrombin-antithrombin complex (TAT, 31.7 µg/L), D-dimer (7.14 µg/mL), and fibrin degradation product (FDP, 82.3 µg/mL). The levels of blood urea nitrogen (BUN) and creatinine were within the normal ranges (25 mg/dL and 0.3 mg/dL, respectively). Neither Shiga-like toxin nor *E. coli* O157:H7 was detected in her stool. However, she had proteinuria, hematuria, and marked petechiae on her body due to thrombocytopenia. She was tentatively diagnosed with HUS, and she received five units of platelet transfusion. Soon after completing the platelet transfusion, she developed generalized convulsions followed by right hemiplegia, and therefore, PE therapy was immediately instituted. On the following day, both CT and MRI examinations of her brain revealed a diffuse hemorrhagic infarction in the left posterior region. The PE therapy was continued for the next 37 days on a total of 19 occasions, along with steroid pulse therapy and high-dose intravenous immunogloburin (IVIG) infusions until clinical improvements were noted. The ADAMTS13:AC and ADAMTS13:INH titers measured by the VWFM assay were less than 3% and greater than 100 BU/mL, respectively (later, both values were re-evaluated by the chromogenic act-ELISA using deep-frozen plasmas, and the respective data were

less than 0.5% and greater than 100 BU/mL [58]). Now, almost 10 years have passed, and the patient is apparently healthy with a minimal sequela of the right hemiplegia.

Case 2

In July 2000, a 19-month-old girl presented with a fever and cough. The next day, she was taken to a family doctor and then transferred to a nearby hospital because of thrombocytopenia (platelet, 21×10^9/L). Upon admission, her laboratory data revealed slight anemia (Hb, 11.3 g/dL), thrombocytopenia (platelet, 38×10^9/L), and an elevated LDH level (994 IU/L). An analysis of a bone marrow aspiration showed no abnormalities, and therefore she was suspected to have ITP. She was administered high-dose IVIG with steroid therapy but her platelet count did not increase. Her platelet count slightly increased soon after the platelet transfusions, while the number of schistocytes in the blood films gradually increased. This patient never had renal dysfunction or neurological signs. Thus, her physician suspected that the patient had USS but did not measure ADAMTS13:AC. The patient was given an infusion of 80 mL of FFP, but her platelet count did not increase. During this period, she was alert and no clinical deterioration was noted. Three months after admission, plasma samples from this patient were sent to our laboratory for ADAMTS13:AC and ADAMTS13:INH testing. Based on the results of the VWFM assays, the patient was diagnosed with a severe ADAMTS13:AC deficiency (< 3%) with ADAMTS13:INH (4.0 BU/mL). However, in those days we were unable to clearly determine whether this patient had USS and developed alloantibodies or acquired TTP with autoantibodies to ADAMTS13. The patient was not given PE therapy because she did not show any clinical deterioration during the subsequent 3 months. Therefore, she was discharged and then carefully observed at the outpatient clinic. However, 1 month after discharge, she was re-admitted to the hospital and received PE therapy because of exacerbated anemia and thrombocytopenia. However, her clinical signs did not improve, even after whole blood exchange therapy. Thus, she was treated with RBC and platelet transfusions, but 2 weeks later she fell into coma and died of tracheal bleeding, which was 8 months after her first hospital admission (plasma ADAMTS13:AC and ADMTS13:INH in this patient were measured only on one occasion. In recent years, both values were re-evaluated by the chromogenic act-ELISA using deep-frozen plasma samples, and the data were less than 0.5% and 4.3 BU/mL, respectively).

Case 3

In July 2002, a 19-month-old boy developed a low-grade fever and cough followed by petechiae. He was taken to a family doctor because his nasal bleeding did not stop. The doctor noted thrombocytopenia and anemia and suspected HUS, and the patient was transferred to a local hospital. He had mild thrombocytopenia (platelet, 70×10^9/L) soon after birth, which spontaneously improved. Upon admission, he was drowsy and his laboratory data showed anemia (Hb, 5.4 g/dL), thrombocytopenia (platelet, 9×10^9/L), elevated LDH (1991 IU/L), and proteinuria. He was administered FFP infusions, steroid therapy, and IVIG. A platelet transfusion was performed but his platelet counts did not significantly increase. Since he was negative for DIC markers, the patient was clinically diagnosed with TTP and then administered PE therapy. After three consecutive PE therapies, he became alert, recovered, and his laboratory markers returned to normal levels. The ADAMTS13:AC and ADAMTS13:INH titers were measured by classic VWFM assays using frozen plasma that was obtained before the PE therapy was administered, and the results were less than 3% and 2.3 BU/mL, respectively (later, chromogenic act-ELISA gave values of less than 0.5% and 2.3 BU/mL, respectively). His plasma ADAMTS13:AC deficiency with ADAMTS13:INH continued for more than 6 months but without appreciable clinical manifestations. After 4 years, ADAMTS13:AC (77%) had normalized and ADAMTS13:INH (< 0.5 BU/ml) was absent.

Case 4

In June 2002, a 13-month-old boy developed a fever followed by dark urine and diarrhea. He was taken to a nearby clinic, where he was determined to have leukocytosis (WBC, 12,000/μL), anemia (Hb, 7.2 g/dL), and thrombocytopenia (platelet, 46×10^9/L). In addition, a peripheral blood film showed phagocytosis and therefore the patient was diagnosed with suspected HPS. He was transferred to a university hospital where platelet transfusions were performed twice for two consecutive days, but his platelet counts only transiently increased. Soon after the second platelet transfusion, a bone marrow aspiration was performed, but the HPS diagnosis was not confirmed. On the other hand, there was a transient increase in his platelet count (platelet, 40×10^9/L) after he was infused with a small amount of FFP, and therefore the physician suspected a diagnosis of USS. Therefore, the patient received a daily plasma infusion therapy for the next 5 days. However, hematuria developed followed by right hemiplegia. An MRI revealed a hemorrhagic infarction (3 × 4 cm) in the left parieto-occipital region. Based on the clinical course, he was eventually diagnosed with ai-TTP. After he received PE therapy for two consecutive days with orally administered prednisolone, his clinical conditions rapidly improved and his laboratory findings recovered. After his recovery, the ADAMTS13:AC and ADAMTS13:INH levels were tested using the classic VWFM assay and deep-frozen plasma samples that were obtained before the PE therapy, and the results were less than 3% and 1.9 BU/mL, respectively (later, these values were re-examined with the chromogenic act-ELISA, and the results were less than 0.5% and 1.9 BU/mL, respectively). He subsequently improved and was discharged. Three years later, his plasma

TABLE III

Comparison of clinical features and outcomes between childhood and adulthood patients with acquired idiopathic (ai)-TTP

Ai-TTP (n = 195)	Childhood patients (n = 14)	Adulthood patients (n = 181)
Age at the onset of TTP bouts (years old), Median (25, 75 percentile)	11 (1.6, 14)	57 (41, 65)
Female (%)	35.7	55.2
"Pentad"		
(1) Platelet count ($\times 10^9$/L), Median (25, 75 percentile)	15 (8, 24)	10 (7, 17)
(2) Hemoglobin (g/dL), Median (25, 75 pecentile)	8.0 (5.6, 8.8)	7.5 (6.3, 8.8)
(3) Renal involvement (%)	71.4	75.7
Serum creatinine (mg/dL), Median (25, 75 percntile)	0.5 (0.3, 0.9)	1.0 (0.7, 1.3)
(4) Central nervous system involvements (%)	71.4	79.6
(5) Fever ($\geq$ 37.0 °C) (%)	92.9	69.6
Times of plasma exchange	5.5 (3.0-17.5)	ND
Mortality in the current episode of TTP bouts (%)	7.1	15.5

ND: not determined.

ADAMTS13:AC had normalized. At present, he has fully recovered and has no residual right hemiplegia.

Case 5

In January 2005, a 9-month-old boy with generalized petechiae and a fever was referred to a local hospital, where he was determined to have thrombocytopenia (platelet, 9×10^9/L). He was admitted to a university hospital and diagnosed with acquired TTP based on ADAMTS13:AC (< 3%) and ADAMTS13:INH titers (2.8 BU/mL) that were determined using the classic VWFM assays. (Later, these values were re-examined by the chromogenic act-ELISA, and the results were less than 0.5% and 4.8 BU/mL, respectively). After he was diagnosed with ai-TTP, he was administered PE therapy for six consecutive days at a different hospital. His clinical symptoms rapidly improved, but the increase in platelet counts was only transient and his platelet count was consistently lower than 10×10^9/L. To prevent serious bleeding complications, the physician administered oral prednisolone, together with continuous low-dose platelet transfusions. Two months later, he was discharged, despite having an ADAMTS13:AC deficiency with ADAMTS13:INH that lasted for at least 8 months. Two years later, we were able to examine the plasma ADAMTS13:AC and ADAMTS13:INH in this patient, and determine that ADAMTS13 had fully normalized [59].

Table III compares the clinical features and outcomes of the childhood patients (n = 14) and adulthood patients (n = 181) with ai-TTP in our registry [13].

Connective tissue disease-associated thrombotic thrombocytopenic purpura

In 1939, Gitlow and Goldmark [60] first reported a close relationship between 'classic' TTP and systemic lupus erythematosus (SLE). In 1999, Brenner et al. [61] described five patients with childhood-onset 'classic' TTP and reviewed 30 other patients who were previously described in the literature. According to their analysis, nine (9/35, 26%) fulfilled four or more ACR criteria for SLE and eight (8/35, 23%) had incipient SLE. Interestingly, of the five patients who were initially diagnosed with 'classic' idiopathic TTP in their laboratory, three were diagnosed with SLE within 3 years, and the remaining two patients fulfilled the ACR classification criteria for SLE within 4 years of disease onset. However, at that time, ADAMTS13:AC assays were not generally available, and therefore no data on ADAMTS13 was provided in their report.

In our registry of 919 patients with TMAs, 221 had CTD-associated TMA, of which 92 had SLE-associated TMA [13]. For the 221 CTD-TMA and/or 92 SLE-TMA patients, the number of patients with a severe ADAMTS13:AC deficiency with ADAMTS13:INH was 46 and 24, respectively. Furthermore, within the 221 patients with CTD-TMA, 11 developed the disease in childhood (less than 15 years of age), including eight patients with SLE, 1 with RA (rheumatoid arthritis), and two with MCTD (mixed connective tissue disease), in whom three had a severe ADAMTS13:AC deficiency. These three patients included two SLE- and one MCTD-associated TTP patients, and they uniformly had relatively low titers of ADAMTS13:INH (0.7–1.8 BU/mL) at

the onset of TTP, which slightly differed from those with ai-TTP (table II). Here we briefly describe these three childhood patients with CTD-associated TTP (cases 15–17) due to their relevance in clinical practice.

Case 15

In April 2005, a 7-year-old girl was determined to have proteinuria and occult blood in her urine based on a school health examination. The patient was admitted to a nearby university hospital for further examination in June 2005, where she was diagnosed with SLE (Lupus nephritis) based on her clinical manifestations and the following laboratory findings: proteinuria, positive for anti-nuclear antibodies and anti-double stranded DNA antibodies, and low complementemia. She was treated with prednisolone, mizoribine, and azathioprine. Because her clinical signs significantly improved with these treatments, she was discharged in September 2005.

In April 2006 (8 years of age), this patient noticed proteinuria and hematuria based on a self-examination at home. The next day, she was admitted to the same hospital. Her laboratory data at the second admission were as follows: Hb (8.8 g/dL), LDH (1608 IU/L), platelet (2×10^9/L), PT (11.0 sec), PTT (31.1 sec), fibrinogen (355 mg/dL), antithrombin (140%), TAT (9.06 µg/L), D-dimer (5.25 ng/mL), FDP (7.9 µg/mL), and schistocytes in a peripheral blood smear. She had a DIC score of 6 according to the DIC diagnostic criteria from the International Society of Thrombosis and Haemostasis [15]. As a result, she was initially treated with nafamostat, but there were no clinical improvements. ADAMTS13:AC and ADAMTS13:INH assays were performed for a differential diagnosis of TTP, and the results were less than 0.5% and 1.2 BU/mL, respectively. Thus, she was diagnosed with SLE-associated TTP and treated with PE (five times), steroid pulse therapy, and cyclophosphamide. These treatments saved her life, and to date, she has not had a relapse of TTP.

Case 16

In July 2006, an 11-year-old girl developed general fatigue, headache, and vomiting. Two days later, she was admitted to a local hospital where laboratory tests indicated the following: Hb (11.9 g/dL), LDH (1636 IU/L), total bilirubin (9.3 mg/dL), platelet (33×10^9/L), PT (12.7 sec), PTT (34.0 sec), fibrinogen (290 mg/dL), antithrombin (> 75%), D-dimer (< 2 ng/mL), FDP (< 5 µg/mL), BUN (18.0 mg/dL), and schistocytes in a peripheral blood smear. For a differential diagnosis, her plasma ADAMTS13:AC and ADAMTS13:INH levels were determined to be less than 0.5% and 1.8 BU/mL, respectively. In addition, upon admission she simultaneously had Raynaud's phenomenon and was positive for anti-nuclear antibodies and anti-RNP antibodies. Thus, she was diagnosed with MCTD-associated TTP, and was administered PE with steroid pulse therapy starting on the third day of hospitalization. During the third PE, she had anaphylactic shock, perhaps related to the infused plasma. Thus, she stopped the PE therapy and continued the steroid pulse therapy alone. As a result of these treatments, she recovered and on the hospital day 14 her ADAMTS13:AC increased to 67% of normal and ADAMTS13:INH became negative. To date, she has had no episodes of TTP.

Case 17

In October 2007, a 12-year-old girl suddenly developed jaundice with a fever. She was admitted to a nearby university hospital, and her routine laboratory data provided the following: Hb (5.6 g/dL), platelet (1×10^9/L), PT (14.0 sec), PTT (40.1 sec), fibrinogen (333 mg/dL), FDP (20.5 µg/mL), total bilirubin (5.5 mg/dL), and schistocytes in a peripheral blood smear. Upon admission, she had low levels of complement, and was positive for anti-nuclear antibodies, anti-double stranded DNA antibodies, and anti-SS-A antibodies. Plasma ADAMTS13:AC and ADAMTS13:INH were simultaneously measured by the act-ELISA and were less than 0.5% and 0.7 BU/mL, respectively. Based on these results, she was diagnosed with SLE-associated TTP and PE therapy was initiated. After three consecutive PE treatments with steroid pulse therapy, her platelet count increased. However, on hospital day 8, her platelet count decreased again, and her ADAMTS13:INH titer increased to 2.2 BU/mL. The PE therapy was re-initiated with steroid pulse therapy. A total of nine rounds of PE therapy and two courses of steroid pulse therapy resulted in remission on hospital day 23. At this time, ADAMTS13:AC and ADAMTS13:INH were 86% and less than 0.5 BU/mL, respectively. To date, she has had no TTP relapses.

Treatment of acquired thrombotic thrombocytopenic purpura

Plasma exchange (PE) is the first line therapy that was demonstrated to be effective in randomized clinical trials for acquired TTP [62]. PE removes ADAMTS13:INH, UL-VWFM, and hazardous cytokines from the circulation in TTP patients, and replenishes ADAMTS13 without circulatory overload. Corticosteroids are often used as an adjunctive treatment. In relapsing or refractory cases, other immunosuppressive drugs such as cyclosporine, cyclophosphamide, vincristine, and rituximab are empirically used. PE therapy should be initiated as soon as possible after TTP is diagnosed, but the onset of therapy tends to be delayed in childhood patients with acquired TTP because of difficult differential diagnoses, especially with HUS, unless ADAMTS13:AC is measured.

In regard to platelet transfusions in TTP patients with a severe ADAMTS13:AC deficiency, these transfusions have consistently been viewed as a contraindication because they may enhance thrombotic complications due to platelet aggregation and thrombus formation under high shear stress generated in the microvasculature. Our experience also partially supports this concept, but such adverse effects happened only on very few occasions. We believe that prophylactic platelet

transfusions should be avoided in TTP patients with a severe ADAMTS13:AC deficiency, but that platelet transfusions must be done if patients experience overt bleeding.

In our 17 childhood patients with acquired TTP, 15 patients were promptly treated with PE and corticosteroid therapy, and 16 children (94%) achieved a first remission. Recently, McDonald et al. [63] reported that the number of PE courses to first remission was higher in children (median, 22.5; range, 10–30) than in adults (median, 15.5; range, 3–93) [64], suggesting that childhood TTP may be more resistant to treatment. By contrast, our results indicated that patients with acquired TTP and a severe ADAMTS13:AC deficiency responded well to PE (median number of PE courses, 5.5; range, 2–39), but two patients (2/17, 11.8%) relapsed and one (1/17, 5.9%) died. Furthermore, in this study, we observed that the children with a high ADAMTS13:INH titer (> 5 BU) tended to require more frequent PE courses to achieve remission.

Fakhouri et al. [65] recently reported that adulthood TTP patients with high-titer ADAMTS13:INH could be successfully treated with a combination of PE and rituximab, a chimeric monoclonal antibody to CD20. The efficacy of rituximab in such patients is apparently due to a reduction in anti-ADAMTS13 IgG antibodies by depleting the patient's B-lymphocytes [65,66]. Recently, there have been many successful cases [67–69], and to date, no significant adverse effects have been reported. In our registry, only one childhood TTP patient (7 years old) with acquired TTP with ADAMTS13:INH was successfully treated with PE followed by rituximab, as shown in table II. However, the best choice or combination in regard to immunosuppressants for treating children with acquired TTP and a severe ADAMTS13:AC deficiency needs to be carefully determined in future studies.

Conclusion

The discovery of ADAMTS13 provided a breakthrough in our understanding of the mechanism of platelet thrombus formation under high shear stress and directly linked this enzyme to TTP pathogenesis in humans. Subsequently, the recent development of rapid and sensitive ADAMTS13 assays and their utilization in clinical practice have shown that the early- and late-onset phenotypes of USS are not different diseases and are likely affected by both acquired endogenous and exogenous circumstances. Furthermore, we have presented a novel category of ai-TTP that occurs during very early childhood (less than 2 years of age), which was perhaps totally overlooked or misdiagnosed before 2002 [39]. Thus, TTP should be recognized as a life-threathening generalized disease that not only occurs in adulthood, but also in childhood, causing a paradigm shift in our clinical understanding of TTP since the first discovery by Moschcowitz in 1924.

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