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

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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 fre-

quent 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

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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 in-

hibitor 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.nih.gov/ni-image/>). Plasma anti-ADAMTS13 IgG antibody titers (binding antibody) were determined by TECHNOZYME® 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.

Fig. 1. Pedigree and ADAMTS13 analysis of USS-LL4 and her family. **A** The proband (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 $141 \times 10^9/l$. 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 $200 \times 10^9/l$. 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 200×10^9 /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 $>50 \times 10^9$ /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.

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Disclosure Statement

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