Type 2B von Willebrand Disease: Early Manifestation as Neonatal Thrombocytopenia

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Abstract

Here, we report about a preterm female newborn with a prolonged course of severe thrombocytopenia and hematomas. The family history was positive for von Willebrand disease type 2B (VWD 2B). Diagnosis of VWD 2B was identified analyzing von Willebrand factor (VWF) parameters (VWF:antigen, VWF:activity, VWF multimer analyses) and performing light transmission aggregometry (with half concentration of ristocetin). In addition, the diagnosis was confirmed by molecular genetic analysis: identification of a disease-causing missense mutation (Val1316Met) in the *VWF* gene associated with a severe course of VWD 2B, which had been previously reported. Treatment with a VWF-containing plasma concentrate was initiated. Because the combination of prematurity and very low platelet count is often associated with intracranial bleeding, at the beginning platelet concentrates were transfused. Fortunately, the patient did not develop serious bleeding episodes. Interestingly, the patient had a mutation in the *VWF* gene, which had been described to be associated with aggravation of thrombocytopenia especially in stressful situations. Therefore, we replaced venous blood withdrawals by capillary blood samplings when possible and, consequently, we observed an increase of the platelet count after this change in management. At the age of 2 months, the patient was discharged after stabilization of the platelet count without any bleeding signs and without a need of long-term medication.

Keywords
- von Willebrand disease
- thrombocytopenia
- neonate

Introduction

von Willebrand disease (VWD) is the most common inherited bleeding disorder caused by mutations in the von Willebrand factor (*VWF*) gene with a prevalence of about 1%.[1] VWD type 1 (~75% of all cases of VWD) is a quantitative defect. VWD type 2 comprises a variety of qualitative defects of VWF and accounts for 20% of VWD cases.[1] Patients with VWD type 3 (<5%) show a complete loss of VWF.[1]

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VWF is stored in megakaryocytes, platelets, and endothelial cells and assembles to large multimeric proteins.[2] After injury of endothelium, VWF mediates platelet adhesion via binding to the GPIbα subunit of the platelet GPIb-V-IX complex and to collagen fibrils in the subendothelium.[2] In secondary hemostasis, VWF plays a role as carrier protein and protector for coagulation factor VIII (FVIII).[2] Patients with VWD present with a variable bleeding diathesis ranging from mild to severe, life-threatening bleeding episodes depending on the subtype of VWD and the kind of trauma.[1] Diagnostic evaluation is based on individual and family bleeding history, specific laboratory analyses, and molecular genetic analyses.[3] Treatment strategies involve desmopressin, VWF-containing concentrates, and tranexamic acid.[1] Just recently, evidence-based guidelines concerning the diagnosis and management of VWD were published by the American Society of Hematology (ASH), the International Society on Thrombosis and Haemostasis (ISTH), the National Hemophilia Foundation (NHF), and the World Federation of Hemophilia (WFH).[4,5]

VWD type 2B (VWD 2B; ~5% of all VWD cases)[2] is mostly characterized by gain-of-function mutations in the A1 domain (rarely at the C-terminal end of the adjacent D3 domain[6]) causing enhanced platelet GPIb-V-IX receptor binding,[2] spontaneous platelet binding, clearance of VWF multimers from plasma, and consumption of platelets leading to thrombocytopenia.[2] The degree of bleeding diathesis is dependent on the specific mutation and on the presence of pathologic stress situations such as pregnancy, surgery, or infection.[7] Treatment strategies are primarily based on replacement of VWF using VWF/FVIII or VWF concentrates, respectively.[2]

This report describes a newborn with a prolonged course of thrombocytopenia caused by VWD 2B. So far, only single reports have been published describing diagnosis and therapy in newborns with VWD 2B.[8–12] We show that the course of severe thrombocytopenia can be pronounced depending on the kind of VWF mutation and on stressful events (i.e., intravenous blood drawing). In addition, we describe that treatment with VWF and, when needed, platelet concentrate transfusions prevented bleeding complications in this patient. Depending on the kind of mutation, we recommend to rather perform capillary blood sampling instead of stressful venipuncture if possible.

Materials and Methods

Laboratory Analyses

VWF Diagnostics

VWF antigen (VWF:Ag), VWF activity (VWF:activity, INNOVANCE VWF Ac), and VWF multimers were determined as previously described.[13,14] VWF activity, VWF antigen (VWF:Ag), and coagulation factor VIII (FVIII:C) (all Siemens Healthcare Diagnostics, Eschborn, Germany) were measured in sodium citrate plasma using the Behring Coagulation System XP (BCS) according to standard protocols. Standard human plasma (Siemens Healthcare Diagnostics) was used for calibration. Measurements were performed within the routine laboratory analysis. The ratio VWF:activity/VWF:Ag ($\geq$0.7) was calculated. VWF multimers were separated on sodium dodecyl sulfate-agarose gel (1 and 2.2%) and blotted on a PVDF (polyvinylidene fluoride) membrane to assess the high-molecular-weight (HMW) multimers. VWF was determined using appropriate primary and secondary antibodies (Von Willebrand Faktor Rabbit Anti-Human, DAKO, Hamburg, Germany and Goat-Anti-Rabbit IgG HRP Conjugate, Biorad, Feldkirchen, Germany) and 3,3-diaminobenzidine/-cobalt chloride (Bio-Rad, Munich, Germany). Standard human plasma (Siemens Healthcare Diagnostics) was used as control.

Quantification of D-Dimers

D-dimers were quantified in sodium citrate plasma using the INNOVANCE D-Dimer test kit (Siemens Healthcare Diagnostics GmbH, Marburg, Germany) and the Sysmex CS-5100 (Sysmex Corporation, Kobe, Japan) according to the protocol suggested by the manufacturer. The quantity of D-dimers is given in fibrinogen equivalent units (FEU). One mg/mL FEU is the concentration of D-dimers resulting from the degradation of 1 mg/mL fibrinogen (reference level $<$ 0.73 mg/L).

Platelet Aggregometry Analyses

Platelet-rich plasma was prepared from citrated blood. Platelet aggregometry (APACT4) was performed using 0.6 mg/mL ristocetin (American Biochemical and Pharmaceutical LTD, Frankfurt, Germany).

Blood Smear

Blood was collected in ethylenediaminetetraacetic acid (EDTA) blood test tubes. Blood smears were dyed with a May–Grünwald solution (Siemens, München, Germany) using a Hema-Tek 3000 System (Siemens, München, Germany). The dyed blood smears were examined using an Olympus BX53 (Olympus, Tokyo, Japan) microscope with a 40-fold magnification.

Differential Diagnostics

Thrombotic thrombocytopenic purpura: The ADAMTS13 activity determined using an enzyme-linked immunoassay (ELISA; Technoclone, Vienna, Austria) was 84% (reference range 50–110%). The ADAMTS13 antigen levels determined using an ELISA (Technoclone, Vienna, Austria) was 0.33 IU/mL (reference range 0.35–1.2 IU/mL). ADAMTS13 inhibitors were not detected using an ELISA (Technoclone, Vienna, Austria, 11 U/mL, reference range $<$ 16 U/mL).

Immune-mediated thrombocytopenia: Blood of the index patient and her mother were sent to the Centre for Transfusion Medicine and Haemotherapy of the University Hospital of Giessen and Marburg, Germany. No maternal alloantibodies were found.

Genetic analysis: Bernard–Soulier syndrome (BSS) was excluded by molecular genetic analysis (wild-type sequences in the *GP1BA*, *GP1BB*, *GP9*, and *FERMT3* genes).

Patient Presentation and Results

Patient Presentation

A 3-day-old female premature newborn was transferred to the neonatal intensive care unit at another hospital because of multiple hematomas and severe thrombocytopenia of 11 G/L (age-adjusted reference range 197–673 G/L). She had been born to a 38-year-old mother (gravida 2, para 2) by secondary cesarean section because of premature labor and rupture of membranes (gestation of 34 weeks). Except for the mother's gestational diabetes mellitus, gestation had been unremarkable. Family history revealed that the patient's father suffers from VWD 2B (which had not been genetically confirmed before). The patient's two paternal half-brothers also presented with postnatal hematomas; however, so far, they have not been evaluated for VWD 2B (►Fig. 1).

After birth, the newborn had shown normal adaptation and normal vital signs. Apgar scores were normal. The birth weight was 2.46 kg (49th percentile), the length 52 cm (>97th percentile), and the head circumference 33 cm (64th percentile).

On day 3, she presented with multiple hematomas at the left shoulder and above the spinous processes. Otherwise, examination was unremarkable.

Cranial and abdominal ultrasound examinations were normal.

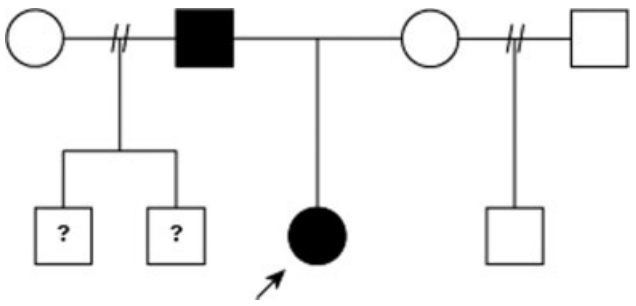

Fig. 1 Family tree of the presented case. The father of the index patient was known to suffer from VWD 2B. The two paternal half-brothers of the index patient were reported to have suffered from hematomas after birth, but were never tested for VWD 2B.

Results

Laboratory Results and Therapy

Platelet count was decreased (11 G/L) and D-dimers were elevated (2.37 mg/L, reference range < 0.73 mg/L). Values for prothrombin and partial thromboplastin time were normal. Because of severe thrombocytopenia, platelets were transfused once at 3 days of age and platelet count increased to a maximum of 93 G/L at the following day; however, it dropped again at postnatal day 5. Ultrasound examinations did not show any cerebral hemorrhage. Further coagulation studies showed reduced levels for VWF activity (44% at postnatal day 4, reference range 50–200%), whereas VWF antigen (112% at postnatal day 4, reference range 50–200%) and factor VIII activity (FVIII-activity, 110% at postnatal day 4, reference range 60–180%) were normal. Because of suspected VWD 2B, substitution with VWF-containing FVIII concentrate was initiated (20 IE/kg body weight daily). For further diagnostic evaluation and therapy, the patient was transferred to our hospital at postnatal day 6. At admission to our hospital, the initial hematomas already began to fade. New hematomas were identified on the back of the right hand and at both heels after blood withdrawals.

After transfer to our hospital, further diagnostic evaluation was pursued. Complete blood count was obtained and showed severe thrombocytopenia (25 G/L). ►Fig. 2 shows the course of the platelet count. Peripheral blood smear showed

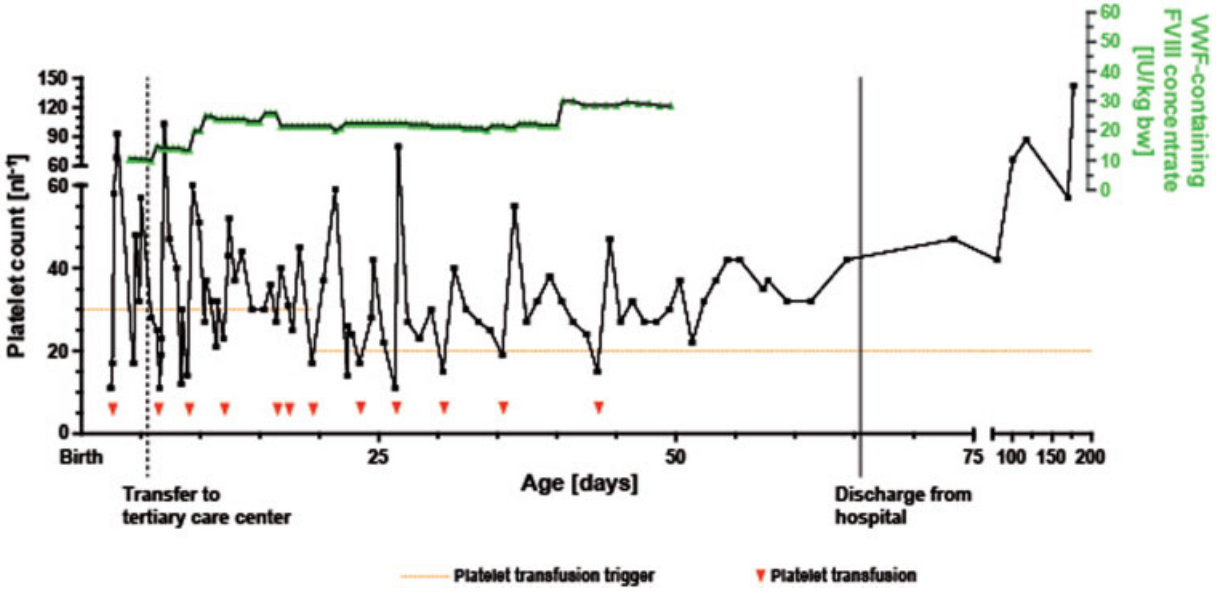

Fig. 2 Course of platelet count in G/L (*black squares*, left y-axis) and substitution of VWF-containing FVIII concentrate (*green triangles*, right y-axis). The *orange dotted line* indicates the platelet count at which transfusion of platelet concentrate was initiated. *Red triangles* indicate platelet transfusions.

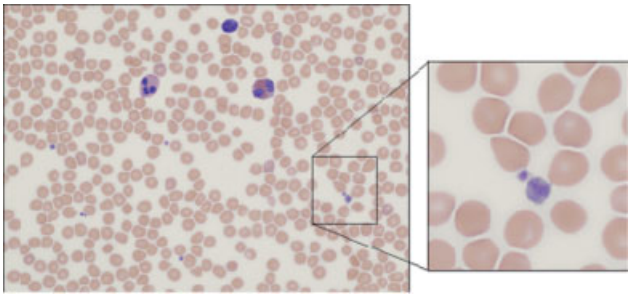

Fig. 3 Peripheral blood smear showing macrothrombocytopenia (see magnification on the right side).

severe thrombocytopenia and macrothrombocytopenia (►Fig. 3). Hemoglobin level was reduced (12.8 g/dL, range 13.7–21.0 g/dL), possibly also because of multiple blood withdrawals. Coagulation factor analyses showed reduced VWF activity levels (40% at postnatal day 7), whereas VWF antigen was normal (146% at postnatal day 7). Multimeric analysis of VWF revealed loss of HMW multimers (►Fig. 4). Using light transmission aggregometry, stimulation with half concentration of ristocetin induced near-maximum platelet agglutination (98%, ►Fig. 5). D-dimers were markedly elevated. At postnatal day 7, D-dimers rose to 26.49 mg/L.

To confirm the diagnosis of VWD 2B, molecular genetic analysis was performed and revealed a disease-causing heterozygous missense mutation in exon 28 of the *VWF* gene c.[3946G > A];[=] p.(Val1316Met) in the patient and her father, which had been previously described. The degree

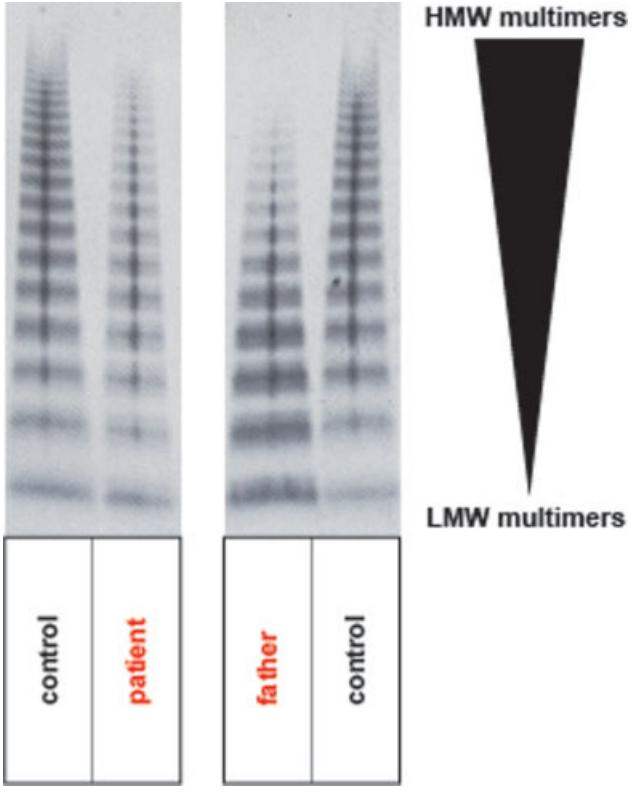

Fig. 4 Multimeric analysis of von Willebrand factor (VWF) in plasma from the index patient, her father, and a control person. HMW: high molecular weight. LMW: low molecular weight.

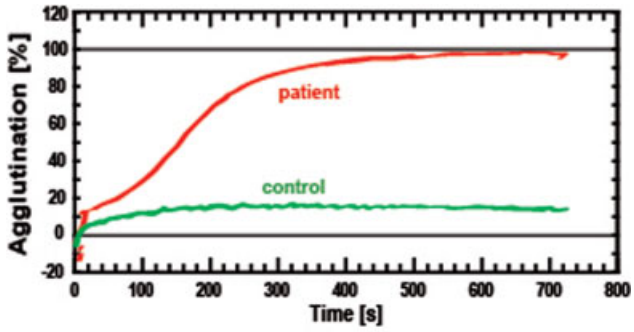

Fig. 5 Ristocetin-induced platelet aggregometry (RIPA): maximal agglutination is reached with half concentration of ristocetin.

of thrombocytopenia, existence of macrothrombocytopenia and platelet aggregates, loss of HMW multimers, and enhanced aggregation of platelets with half concentration of ristocetin are typical features in patients with VWD 2B.[7] The Val1316Met variant in the *VWF* gene is associated with pronounced thrombocytopenia, platelet aggregates and giant platelets (blood smear), loss of HMW multimers, and enhanced agglutination of platelets.[7] ►Table 1 shows diagnostic procedures usually performed in the case of suspected VWD 2B, expected results in VWD 2B (third column), and results obtained in this case (fifth column).

Discussion

Differential diagnosis of neonatal thrombocytopenia is wide and can be divided in pathologies with decreased production of platelets and in those with increased consumption of platelets.[15] In this case, the positive family history hinted at VWD 2B. Nevertheless, other etiologies of neonatal thrombocytopenia were investigated, additionally. Maternal alloantibodies were negative excluding immune-mediated forms of thrombocytopenia. Normal activity of the plasma metalloproteinase ADAMTS13 ruled out neonatal thrombotic thrombocytopenic purpura. Platelet-type VWD and BSS were excluded by genetic analysis showing wild-type alleles for the corresponding genes: platelet-type VWD, an inherited platelet disorder caused by a gain-of-function mutation in the platelet GP1BA receptor, mimics clinical presentation of VWD 2B. BSS is caused by loss-of-function mutations in the *GP1BA*, *GP1BB*, or *GP9* genes and patients with BSS present with bleeding diathesis and macrothrombocytopenia.

Patients with VWD 2B are at increased risk of suffering bleeding complications.[2] Bleeding diathesis results from selective loss of HMW multimers and thrombocytopenia.[2] Bleeding severity also depends on the localization of the mutation within the gene.[7] For example, Casari et al showed that the bleeding diathesis of VWD 2B caused by the Val1316Met mutation, which was found in our patient, is also partly attributable to platelet dysfunction caused by altered protein kinase C signaling.[16,17] Federici et al described a cohort of patients with VWD 2B with different mutations showing that the here-identified Val1316Met mutation is associated with a lower baseline platelet count and a loss of HMW and intermediate-molecular-weight multimers leading to a higher

Table 1 Selection of laboratory tests for the case of suspected von Willebrand disease type 2B (VWD 2B)

Laboratory assays	Reference range age-adjusted	Expected test results in VWD 2B	Expected test results in VWD 2B with a Val1316Met mutation	Test results of the index patient (with a Val1316Met mutation) with the time point of laboratory test in days after birth given in square brackets
VWF activity (VWF:activity)	50–200%	↓	↓ ↓	40% (50–200%) [7]
VWF antigen assay (VWF:Ag)	50–200%	Normal/↓	Normal/↓	146% [7]
VWF:activity/VWF:antigen	0.73–1.16	<0.6	↓ ↓	0.27 [7]
FVIII activity (FVIII:C)	60–180%	Normal/↓	Normal/↓	147 [7]
Platelet count	197–673 G/L	Normal/↓	↓ ↓	↓ ↓ ↓
Blood smear	Normal	Normal/ abnormal	Platelet aggregates, macrothrombocytopenia	Platelet aggregates, macrothrombocytopenia [8]
VWF multimer analysis	Normal	Normal/ abnormal	Loss of HMWM	Loss of HMWM [4]
RIPA with half concentration of ristocetin	Low	↑↑	↑↑↑	↑↑↑ [12]

Abbreviations: HMWM, high-molecular-weight (HMW) multimers; RIPA, ristocetin-induced platelet aggregometry; VWD 2B, von Willebrand disease type 2B; VWF, von Willebrand factor.
Note: Expected test results in VWD 2B (third column), expected test results in VWD 2B with a Val1316Met mutation (fourth column), and test results of the index patient (fifth column).

bleeding tendency and longer bleeding time.[7] Furthermore, stress situations (i.e., surgery, infection) can aggravate thrombocytopenia, which can secondarily increase the risk of bleeding.[7] There are only few case reports of newborns affected by VWD 2B.[8–12] Premature newborns with VWD 2B may be at increased risk of hemorrhagic complications (i.e., intracerebral bleeding). Therefore, we continued treatment with VWF-containing FVIII (20–50 IE/kg body weight daily) (►Fig. 2). VWF activity levels were closely monitored (►Fig. 6). Initially, platelet concentrate transfusion was performed if platelet count dropped below 30 G/L. After 2 weeks, when further bleeding signs had not occurred, the threshold value for platelet concentrate transfusion was lowered to 20 G/L. Platelet count always increased after transfusion and climbed to a maximum of 103 G/L; however, it usually dropped again during the following days (►Fig. 2). Head ultrasound examination remained normal. As stressful situations are known to aggravate thrombocytopenia in patients with the here-identified Val1316Met variant in the *VWF* gene,[7] we tried to avoid as many venous blood withdrawals as possible and performed mainly capillary whole blood counts.

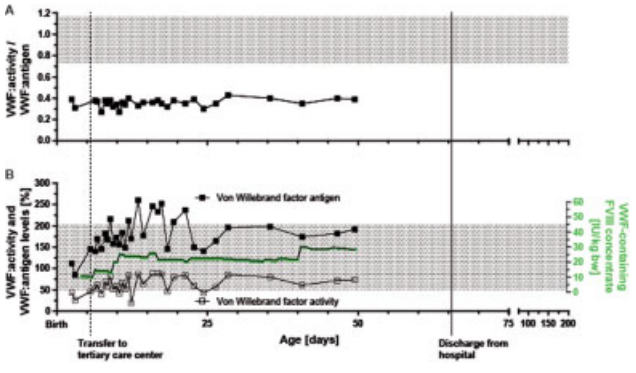

Fig. 6 Course of (**A**) decreased ratio of VWF:activity to VWF:antigen and (**B**) VWF:activity and VWF:antigen levels (*unfilled* and *filled squares*, respectively, left y-axis). (**B**) VWF-containing FVIII concentrate in *international units (IU) per kilogram (kg) body weight per day (d)* (*green triangles*, right y-axis) until 200 days of age.

As platelet counts remained stable between 30 and 40 G/L, regular infusions of VWF concentrates were discontinued. Platelet counts remained stable. Finally, the girl was discharged from our hospital without any bleeding signs and without any need for long-term medication. During the following months, the girl developed normally, showed increasing platelet counts achieving 199 G/L (at the age of 8 months), and did not develop further bleeding episodes.

Literature concerning management of VWD 2B in the neonatal period is restricted to few case reports and expert opinions.[8–12,18] In all but one cases reported in the literature, the mothers of the affected newborns had already been known to suffer from VWD 2B.[8–11,18] Therefore, blood was withdrawn immediately after birth, revealing severe thrombocytopenia between 4 and 19 G/L.[8–11] All newborns from mothers with a known VWD 2B received VWF-containing blood products and platelet transfusions directly after birth.[8–11] Fan et al report the case of a newborn with severe thrombocytopenia and intracranial hemorrhage, which was first attributed to immune thrombocytopenia and therefore treated with platelet transfusions, intravenous immunoglobulins, and corticosteroids. Only at the age of about 2 months, the diagnosis of VWD 2B was made by identification of a de

novo Val1316Met mutation in the *VWF* gene.[12] McLaughlin and Kerr reported on a male baby born by cesarean section who presented with a significant cephalhematoma after birth, which resolved quickly after administration of platelets and VWF concentrate.[11] Proud and Ritchey describe a male newborn with the same V1316M variant as our patient. Because of frequent transfusions of platelet concentrates, he received a central catheter. Despite preoperative VWF concentrate infusion, the patient developed a rapidly expanding hematoma at the surgical site, which stopped only after transfusion of platelet concentrate. They reported that the most challenging question was determining the length of treatment as there are no specific guidelines. At the age of 2 months, the central access catheter was removed.[9]

In summary, we present a prematurely born girl with manifestation of VWD 2B on day 3 of life. Existing guidelines on the management of VWD 2B are based on results derived from nonrandomized studies and mainly descriptive studies.[2] We continued treatment until the age of 2 months when thrombocytopenia stabilized above 40 G/L. Stressful events should be avoided, if possible, because of aggravation of thrombocytopenia. Therefore, we used capillary blood sampling because these are less invasive and therefore less stressful than venipuncture.

Conflict of Interest

The authors declare that they have no conflict of interest.

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