Acquired Von Willebrand Syndrome (AVWS) Type 2A as the Presenting Feature of JAK2 Wild type Thrombocythemia in a Child: Effectiveness of Platelet Reduction by Anagrelide

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Abstract

A 9-year-old Caucasian boy presented in 1994 with severe headache, attacks of migraine, aggressive behavior and minor bleeding symptoms. Initial abnormal laboratory data were a platelet count of 1596 × 10^9/L and slight splenomegaly on echogram. Low-dose aspirin 100 mg/day relieved the cerebral symptoms, but a pronounced spontaneous bleeding tendency became evident. Severe epistaxis, bruises, hematomas and gum bleedings resulted in an iron deficiency state (hemoglobin 5.7 mmol/L, hematocrit 0.30, MCV 77 fl, ferritine 6 ug/l) in November 1995. The combination of mucocutaneous bleeding, high platelet counts (1946 × 10^9/L), and increase of enlarged megakaryocytes in a bone marrow smear was consistent with the diagnosis of hemorrhagic thrombocythemia. Coagulation studies revealed the presence of an acquired von Willebrand syndrome type 2A featured by a prolonged IVV bleeding time, near normal factor VIII coagulant activity (0.53 u/ml) and von Willebrand factor (VWF) antigen (0.55 u/ml), low VWF ristocetine cofactor activity (0.29), very low VWF collagen binding activity (0.16 u/ml) and absence of large VWF multimers. A severe epistaxis lasting for several hours stopped immediately after correction of the VWF parameters by substitution of 3000 units. Bone marrow histopathology showed a pronounced increase of large immature megakaryocytes with hyperlobulated nuclei and maturation defects of nuclei and cytoplasm, and absence of reticuline fibrosis consistent with the diagnosis of JAK2 wild type thrombocythemia in primary megakaryocytic granulocytic myeloproliferation (PMGM). Initial treatment with hydroxyurea (500 mg daily) followed by anagrelide resulted in correction of platelet count from 2000 × 10^9/L to near normal (400-600 × 10^9/L) by, which was associated with relief of bleeding symptoms, and correction of plasma VWF values and VWF multimeric pattern to normal. The subsequent natural history was featured by spontaneous reduction of platelet count to near normal and no progression of JAK2 wild type myeloproliferative neoplasms for more than 13 years follow-up.

Keywords Hemorrhagic thrombocythemia; Myeloproliferative neoplasm; JAK2 wild type; Acquired Von Willebrand Disease; Essential thrombocythemia; Hydroxyurea; Anagrelide

Introduction

Essential thrombocythemia (ET) with either thrombotic or bleeding manifestations as the presenting symptoms of a myeloproliferative disorder usually occurs at old age above 50 years, but also affects young adults [1-3]. ET is rare in children. Clinical manifestations of microvascular circulation disturbances and/or mucocutaneous bleeding have been reported in about 26 cases with childhood ET until 2000 [4-7]. Philadelphia chromosome negative and bcr negative ET is one of the less rare variants of chronic myeloproliferative disorders [8-11]. According to the criteria of the Polycythemia Vera Study Group (PVSG) [12], ET is a diagnosis of exclusion other variants of myeloproliferative disease. The Thrombocythemia Vera Study Group (TVSG) [13] used bone marrow histology as a specific clue to the diagnosis of ET[1,2]. The use of the PVSG and TVSG criteria for the clinical diagnosis of ET (Table 1) include three types of ET in various myeloproliferative disorders when the bone marrow features according to the 2006-2008 European Clinical Molecular and Pathological (ECMP) criteria are applied (Table 1) [2]. In this study we present a child who presented with aspirin-responsive microvascular circulation disturbances followed by spontaneous hemorrhages due to an acquired von Willebrand syndrome as the presenting feature of JAK2 wild type hypercellular ET in primary megakaryocytic granulocytic myeloproliferation (PMGM) [2].

Methods Hematological Investigations

Hematologic data were obtained using routine procedures. Blood and bone marrow smears were stained by the May-Grünwald-Giemsa method and processed for the periodic acid-Schiff (PAS) reaction. The leukocyte alkaline phosphatase (LAP) score was determined with the cytochemical technic of Hayhoe and Quaglino [14,15]. Bone marrow biopsies were done with a Jamshidi needle and embedded in glycol methylcrylate without decalcification. Sections were stained with hematoxylin-azophloxin, PAS, gallamine-Giemsa, and Gomori’s reticulin method. Cytogenetic analysis followed standard procedure. Nucleated cells from peripheral blood and bone marrow aspirates were cultured for 24 and 48 hours, without addition of phytohemaglutinin but with methotrexate treatment of the cultures. All metaphases were banded with the use of G-, Q-, or R-bands and analysed according to
the International System for Cytogenetic Nomenclature (ISCN) [16]. Southern blot analysis was performed as previously reported [17]. In brief, Bgl II, Hibd III, Bam HI and Eco RI-digested DNA was electrophoresed on a 0.7% agarose gel, blotted, and hybridized to the bcr probes, i.e., the 5’ Bgl II- Hind III bcr fragment and the 3’ Hind III- Bgl II bcr fragment [17]. The hemorrhagic thrombocytopenia as the presenting manifestation of a myeloproliferative disorder was characterized according to the recently defined bone marrow features according to ECMP criteria for the diagnosis of essential thrombocytopenia, polycythemia vera and primary megakaryocytic granulocytic myeloproliferation (PMGM) [2,11,14].

| European Clinical and molecular (ECM) criteria | Bone marrow pathology (P) criteria (WHO) |
|-----------------------------------------------|------------------------------------------|
| JAK2V617F ET | Normocellular ET |
| Platelet count of >350 × 10⁹/L and the presence of large platelets in a blood smear | Predominant proliferation of enlarged mature megakaryocytes with hyperlobulated nuclei and mature cytoplasm, lacking conspicuous morphological abnormalities. |
| **Presence of JAK2V617F mutation** | |
| Normal erythrocytes <5.8 × 10¹²/L males, <6 × 10¹²/L females | No increase, proliferation or immaturity of granulopoiesis or erythropoiesis. |
| Normal haemoglobin (Hb) and hematocrit (ht) | Reticuline fibrosis (RF) 0 or 1 |
| JAK2V617F prodromal PV | ET with bone marrow features of PV |
| Platelet count of >350 × 10⁹/L and normal ht male <0.51, female<0.48, normal erythrocyte <5.8 × 10¹²/L males, < 6 × 10¹²/L females is mandatory. | Increased cellularity with due to increased erythropoiesis or trilineage myeloproliferation (i.e. panmyelosis). Proliferation and clustering of small to giant (pleomorphic) megakaryocytes. |
| **Presence of JAK2V617F mutation** | |
| Low serum EPO level and/or increased LAP score | Absence bone marrow features consistent with congenital polycythemia and secondary erythrocytosis. |
| **Spontaneous EEC.** | |
| JAK2V617F hypercellular ET | JAK2-MGM |
| Platelet count of >350 × 10⁹/L, | Hypercellular ET due to chronic megakaryocytic and granulocytic myeloproliferation (JAK2-MGM) and normal or reduced erythroid precursors. |
| No signs of leuko-erythroblastosis | Loose to dense clustering of more pleomorphic megakaryocytes with hyperploid or clump dysplastic nuclei (not or some cloud-like). |

**Table 1**: The 2008 European Clinical Molecular and Pathobiological (ECMP) criteria for the diagnosis JAK2V617F mutated essential thrombocythemia (ET).

**Coagulation assays**

Hemostatic tests were performed using routine procedures. Bleeding times were measured according to Ivy et al. [18]. Platelets were counted in EDTA blood samples using the Platelet Analyzer 810 (Baker Instruments, Allentown, PA). Platelet aggregation induced by collagen (Sigma Chemical St Louis) in optimal concentration, by ADP (Sigma Chemical St Louis ) 10⁻⁵ M f (final concentration) and 10⁻⁶ M f, by epinephrine (Sigma Chemicals St Louis) 10⁻⁵ M f and 10-6 M f and Factor VIII coagulant activity (FVIII:c) was assayed by means of the Automatic Coagulation Laboratory (ACL; Instrumental Laboratory, Jesselstein, The Netherlands) using FVIII-deficient plasma (Ortho Diagnostic Systems, Beersel, Belgium). Ristocetin (H. Lundbeck Co, Copenhagen, Denmark) induced platelet aggregation (RIPA) was recorded with the use of a serial aggregometer (Payton model 300B: Scarborough, Canada) in platelet-rich plasma at a standard count of 200 × 10⁹/L.

**Von Willebrand factor (vWF) assays**

VWF antigen (VWF: Ag) was assayed by an enzyme-linked immunosorbent assay (ELISA), as described by Czejka [19]. Ristocetin
cofactor activity (vWF: RCo) was assayed with formalin-fixed platelet using a PAP4 Model Aggregometer (Bio-Data Corp. Hatboro, PA) according to MacFarlane et al. [20]. The collagen binding activity of vWF (vWF: CBA) was measured according to the ELISA-based method of Brown and Bosak [21] with slight modifications by Dr Van Vliet of the Rotterdam Clinical Research Laboratory (RCRL). The collagen suspension was prepared by overnight hydration of one gram collagen (bovine achilles tendon, type I, C9879; Sigma Chemicals, St Louis, MO) in 400 ml 3% acetic acid at 4°C and homogenisation for 30 minutes in a blender. According to these procedure pellets, if any, formed after centrifugation at 2500 g for 15 minutes should be very small. The suspension should look silky and fibrous; if it looks granular homogenisation has to be repeated. Buttons and supernatant mixed by stirring were stored at 4°C. Microporifer plate wells (A/S Nunc, Roskilde, Denmark) were coated overnight at 4°C with 100 µl of a 0.2 mg/ml suspension of collagen in 20 mmol/L acetic acid. The wells were emptied, washed with phosphate-buffered saline (PBS)-0/05% Tween-20 (PBS-Tween). Subsequently, 100 µl of 1/40 dilutions of plasma in PBS-Tween-1% albumin were added to the wells and incubated for 2 hours at room temperature. After washing, the wells were incubated with 100 µl of a 1/200 dilution in PBS-Tween-Albumin of horseradish peroxidase-conjugated rabbit polyclonal Igs to human vWF (Dako A/S, Glostrup, Denmark) for another two hours at room temperature. After washing, 100 µl ABTS substrate solution [27.4 mg ABTS (2.2'-azino-di-[3-ethyl-benzthiazolsulphonate(8)] dissolved in 10 ml buffer, containing 10 mmol/L citric acid. 100 mmol/L NaHPO4 and 10 µL 30% H2O2 was added. After 15 minutes of incubation, the color development was stopped by the addition of 10 µl concentrated acetic acid and the extension was measured at 414 nm. The in house vWF: CBA method by Dr van Vliet has not changed in the Rotterdam Clinical Research Laboratory (RCRL) before, during and after the period 1992 to 1998 of the presented study.

The multimeric composition of vWF by Budde (Hamburg Coagulation Lab) was analyzed using sodium dodecyl sulfate (SDS) agarose discontinuous gel electrophoresis according to Ruggeri et al with slight modifications [22,23]. Briefly, the samples were diluted between 1:10 and 1:20, according to their VWF:Ag content using a buffer composed of 10 mmol/L Tris-HCL, 1 mmol/L EDTA, 2% SDS, ph 8.0, and subjected to electrophoresis overnight. The separated multimers were then transferred onto nitrocellulose by electroblootting with a 50 mmol/L phosphate buffer, ph 7.4, containing 0.04% SDS, and incubated sequentially with a rabbit anti-vWF antibody (Dakopatts, Hamburg, Germany) and a goat antirabbit IgG conjugated to horseradish peroxidase (Bio-Rad, Munich, Germany). Detection of the VWF multimers by luminography was achieved by incubating the nitrocellulose membrane with 5 ml of a solution prepared by mixing 40 mg of luminal (Sigma, Munich, Germany) and 10 mg 4-iodophenol (Aldrich, Munich, Germany) into 100 ml of Tris-buffered saline (20 mmol/L Tris-HCL, 500 mmol/L NaCl, ph 7.5). The luminescent blots were then covered by a translucent polyethylene film and exposed to a Y-ray film (X-OMAT S, Kodak, Stuttgart, Germany) for 3 to 5 seconds.

**Criteria for diagnosis and classification of essential thrombocythemia (ET)**

Since 1992 we used the established inclusion, exclusion and confirmative criteria proposed by the TVSG [13] and PVSG [12] for the clinical diagnosis of ET (Table 1). We subsequently apply bone marrow features and European Clinical, Molecular and Pathobiological (ECMP) criteria for the classification of ET in various myeloproliferative disorders [2]. The application of the ECMP classification distinguish three phenotypes of JAK2V617F mutated ET including: normal cellular ET, ET with features of early PV (prodomal PV, and ET associated with prethrombotic megakaryocytic granulocytic myeloproliferation: JAK2-MGM (Table 1).

**Case Report**

A 9-year-old Caucasian boy presented in June 1994 with severe headache, attacks of migraine, aggressive behavior and minor bleeding symptoms. His past medical and familial history were unremarkable. On physical examination the tip of the spleen was palpable. The length diameter of the spleen on ultrasound scan was enlarged, length diameter 10 cm. A MRI-scan of the head and an EEG were non-diagnostic. Initial laboratory data in 1994 were hemoglobin 14 g/dl (8.75 mmol/L), htc 0.38, MCV 86 fl, platelets 1596 × 1012/L, leukocytes 9 × 109/L, a normal white blood cell differential count and LDH 350 u/L. Renal and liver function tests and ferritine (28 ng/L) were normal. While on continuous low dose aspirin 100 mg/day the cerebral symptoms relieved and did not recur in 1994 and 1995, but a pronounced spontaneous bleeding tendency became apparent in 1995. Recurrent severe epistaxis lasting for several hours up to 2 days, recurrent bruises, hematomas and gum bleedings persisted throughout 1995, which ultimately resulted in an iron deficiency state in November 1995 (Hb 5.7 mmol/L, htc 0.30, MCV 77 fl, ferritine 6 ug/L). The clinical picture of microvascular circulation disturbances followed by spontaneous mucocutaneous bleeding manifestations was associated with persistently increased high platelets counts between 1200 and 2200 × 1012/L (Figure 1) in the context of hypercellular essential thrombocythemia (Figure 2).

![Figure 1](https://example.com/figure1.png)

**Acquired von Willebrand Syndrome**

The pronounced increase of platelet count was associated with an acquired von Willebrand syndrome with values for FVIII: C 0.53, VWF: Ag 0.55, VWF: RCo 0.29 and VWF: CB 0.16 U/ml and prolonged Ivy bleeding time (10 to 15 minutes).
Figure 2: Bone marrow features showing a clearly hypercellular bone with prefibrotic primary megakaryocytic and granulocytic myeloproliferation (PMGM) featured by loose to dense clustering of abnormal megakaryocytes, their variations in size, some naked nuclei and no increase of reticulin fibers (bottom middle). There are definite abnormalities of maturation with bulky (bulbous) hyperchromatic nuclei and some disturbances of the nuclear cytoplasmic ratio implying atypia (see arrows right) consistent with PMGM.

A severe epistaxis on November 28, 1995 lasting for several hours stopped immediately after correction of the VWF parameters to normal (VWF: Ag 1.21 U/ml, VWF: RCo 1.14 U/ml, VWF: CB 0.83 U/ml) by substitution of 3000 units Hemate-P (Aventis Behring, Marburg, Germany). Bone marrow biopsy under general anesthesia under correction of VWF parameters by substitution of 3500 units Hemate-P (Aventis Behring, Marburg, Germany) was uneventful and not followed by bleeding complications. Analysis of the VWF-multimers at time of overt hemorrhagic thrombocythemia in November 1995 (platelets 1946 × 10⁹/L, Figure 1) revealed a significant loss of the high-molecular-weight multimers of VWF comparable with type II von Willebrand Disease (Figure 3).

Reduction of platelet count by treatment of hydroxyurea to near normal values between 400 and 600 × 10⁹/L followed by long-term treatment with anagrelide (Figure 1) was associated with no recurrence and absence of bleeding manifestations, correction of plasma levels of VWF: Ag, VWF: RCo and VWF: CB to normal values together with the reappearance of the high molecular-weight VWF multimers (Figure 4).

Diagnosis of JAK2 wild type Thrombocythemia in PMGM

The peripheral blood film showed red cells with slight anisocytosis and microcytosis, a few schistocytes, a few ovalocytes and a sporadic tear drop cell, absence of normoblasts, a normal white blood differential count (metamyelocytes 0.5%, banded forms 1%, segmented granulocytes 52%, basophiles 2.5%, lymphocytes 35% monocytes 6%), and pronounced increase and clumps of platelets. The leucocyte alkaline phosphatase (LAP) score was low 14 (normal score 10–100).
Bone marrow aspirates yielded material with clumps of platelets in two smears and one smear showed normal cellularity with normal erythropoiesis, slightly increased myelopoiesis with normal maturation, and a pronounced increase of enlarged megakaryocytes with large multilobulated nuclei (Figure 2). The iron stain with Prussian blue was negative consistent with the iron deficient state (ferritin 6 ng/ml) due to recurrent bleeding.

Bone marrow biopsy specimens showed a clearly hypercellular bone marrow with a predominating primary megakaryocytic and granulocytic myeloproliferation according to Georgii & Michiels (PMGM)[2,3] and the absence of reticulin or collagen fibers (Figure 2). Noteworthy is the loose to dense clustering of large immature megakaryocytes, their great variations in size with hyperploid nuclei and some naked nuclei. In addition, several megakaryocytes show definite abnormalities of maturation with bulky (bulbous) hyperchromatic nuclei and some disturbances of the nuclear cytoplasmic ratio (Figure 2). These bone marrow findings are consistent with thrombocytathemia as the presenting feature of PMGM (Table 2) [2,3,10,11].

| J.J.Michiels | Clinical Criteria 2005 | J.Thiele | Pathological criteria 2005/2008 |
|--------------|------------------------|----------|-------------------------------|
| A1 | No preceding or allied other subtype of myeloproliferative neoplasm, CML or MDS. Main presenting Features is pronounced Thrombocytathemia JAK2 and MPL wild type | B1 | Primary megakaryotic and granulocytic myeloproliferation PMGM and no or relative reduction of erythroid precursors. Abnormal clustering and increase in atypical giant to medium sized Megakaryocytes containing bulbous (Cloud like) hypolobulated nuclei and definitive maturation defects. |
| C | Clinical stages of PMF or PMGM | MF | Staging of myelofibrosis(MF) |
| C1 | Early clinical stages Normal Hemoglobin or slight anemia, grade 1: hemoglobin> 12 g/dl | MF-0 | Prefibrotic stage PMF or PMGM: no reticulin fibrosis |
| | Slight or moderate splenomegaly on ultrasound scan or CT Thrombocytomegaly, Platelets in excess of 400, 600 or even 1,000×10^9/L | MF-1 | Early PMF or PMGM slight reticulin fibrosis |
| | Normal or increased LAF-Score | - | - |
| | No leuko-erythroblastose | - | - |
| C2 | Intermediate clinical stage | - | - |
| | Anemia grade II:Hemoglobin >10 g/dl | MF-1 | PMF or PMGM: slight reticulin Fibrosis |
| | Definitive leuko-erythroblastic blood pictures and/or tear drop erythrocytes Increased LDH | MF-2 | Fibrotic PMG or PMF: Marked increased in reticulin and slight to moderate collagen fibrosis |
| | Splenomegaly | - | - |
| C3 | Advanced Clinical stage | - | - |
| | Anemia grade III:Hemoglobin <10g/dl | MF-3 | Fibrotic PMG or PMF: advanced collagen |
| | Definitive leuko-erythroblastic blood pictures and/or tear drop erythrocytes | - | - |
| | Splenomegaly, leukocytosis, leukopenia | Thrombocytopenia | - | - |

Table 2: European Clinical Molecular and pathological (EMCP) criteria for diagnosis and staging of PMF or PMGM.

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Discussion

Hemorrhagic thrombocytathemia is defined as a clinical syndrome of spontaneous mucocutaneous hemorrhages associated with extremely high platelet counts in patients with thrombocytathemia of various myeloproliferative disorders [22-30]. Several reports in adults demonstrate that hemorrhagic thrombocytathemia with very high platelet counts in excess of 1500 to more than 6000 × 10^9/L is associated with an acquired von Willebrand factor deficiency [31]. The laboratory features of acquired von Willebrand syndrome with spontaneous bleeding symptoms at time of investigation in reported adults with hemorrhagic thrombocytathemia are characterized by: A very high platelet count (range 1285 to 5860 × 10^9/L), a prolonged Ivy or Simplate bleeding time, a normal or near normal factor VIII coagulant activity and VWF antigen (VWF: Ag) concentration, a very low VWF-ristocetin cofactor activity (VWF: RCo) and vWF-collagen...
binding activity (VWF: CB) and absence of large and some of the intermediate VWF multimers simulating a type II von Willebrand disease [31]. The present study is the first report of a child with a typical clinical picture of hemorrhagic thrombocythemia associated with a well-documented acquired von Willebrand syndrome type 2A. In all adults, reduction of platelet count to near normal resulted in the complete relief of bleeding manifestations, the disappearance of the acquired von Willebrand syndrome, and correction of all VWF parameters to normal with the reappearance of the intermediate and high VWF multimers in plasma (Figure 4) [25-31]. A correct diagnosis in patients with a persistent increase of platelet counts, either asymptomatic or with microvascular thrombotic complication and/or hemorrhagic manifestations, remains a challenge. According to WHO ECMP criteria, bone marrow histopathology appears to be a specific clue to a proper classification and diagnostic differentiation of thrombocythemia in various myeloproliferative disorders [2,32,33]. In reactive thrombocythemia the number of megakaryocytes is increased, but the morphology and size of megakaryocytes remain normal, small and mature and there is no tendency to clustering [2,11,32,33]. In Ph-positive ET and in thrombocythemia associated with Ph-positive chronic myeloid leukemia, the megakaryocytes in bone marrow smears and biopsy material are clearly smaller than normal with round nuclei showing minor lobulations [34]. In contrast, both the number and size of megakaryocytes in bone marrow smears and biopsies are typically increased in Ph-negative ET [34]. Enlarged megakaryocytes with mature cytoplasm and multilobulated nuclei and their tendency to cluster in small groups close to sinuses are the hallmark of JAK2(V617F) ET [2,32,33]. The histological background of hematopoiesis in JAK2(V617F) ET is featured by large mature pleomorphic megakaryocytes and normal cellularity of erythropoiesis and myelopoiesis [2,32,33]. The megakaryocytes in polycythemia vera may have a rather pleiomorphic appearance with a wide range of sizes, including small to giant forms, and the characteristic increase and clustering of enlarged megakaryocytes. Proliferation of erythropoiesis with hyperplasia of dilated sinuses are the diagnostic hallmark of untreated polycythemia vera to distinguish it from secondary reactive polycythemias [2,32-34]. The very characteristic histopathological feature of PMGM3 according to Georgii et al. [3] is a mixed dual proliferation of increased granulopoiesis and megakaryopoiesis without reticulin or collagen fibrosis, but dominated by atypical immature giant megakaryocytes, which are conspicuously enlarged due to increase of nuclear as well as cellular size with bulky and irregular, roundish-shaped nuclei [2,32,33]. Pronounced dysmegakaryopoiesis with immature cytoplasm and with the so-called cloud-like nuclei are typical for early fibrotic and fibrotic stages of PMGM [2,3] which are (almost) never seen in JAK2(V617F) ET or polycythemia vera [2,33].

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