Journal of Hematology & Thromboembolic Diseases

Michiels et al., J Hematol Thrombo Dis 2017, 5:4
DOI: 10.4172/2329-8790.1000274

Research Article OMICS International

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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Received date: July 10, 2017; **Accepted date:** August 03, 2017; **Published date:** August 08, 2017

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 Ivy 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-azophyloxin, 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, Bgb 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 thrombocythemia 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 thrombocythemia, polycythemia vera and primary megakaryocytic granulocytic myeloproliferation (PMGM) [2,11,14].

European Clinical and molecular (ECM) criteria	**Bone marrow pathology (P) criteria (WHO)**
$JAK2^{V617F}$ ET	Normocellular ET
Platelet count of >350 × 10^9/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 $JAK2^{V617F}$ mutation	
Normal erythrocytes <5.8 × 10^{12}/L males, <6 × 10^{12}/L females	No increase, proliferation or immaturity of granulopoiesis or erythropoiesis.
Normal haemoglobin (Hb) and hematocrit (ht)	Reticuline fibrosis (RF) 0 or 1
$JAK2^{V617F}$ prodromal PV	ET with bone marrow features of PV
Platelet count of >350 × 10^9/L and normal ht male <0.51, female<0.48, normal erythrocyte <5.8 × 10^{12}/L males, < 6 × 10^{12}/L females is mandatory.	Increased cellularity with due to icreased erytropoiesis or trilineage myeloproliferation (i.e. panmyelosis). Proliferation and clustering of small to giant (pleomorphic) megakaryocytes.
Presence of $JAK2^{V617F}$ mutation	
Low serum EPO level and/or increased LAP score	Absence bone marrow features consistent with congenital polycythemia and secondary erythrocytosis.
Spontaneous EEC.	**RF 0 or 1**
$JAK2^{V617F}$ hypercellular ET	JAK2-MGM
Platelet count of >350 × 10^9/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 pleiomorphic megakaryocytes with hyperploid or clumpsy nuclei (not or some cloud-like).
Slight or moderate splenomegaly on ultrasound presence of $JAK2^{V617F}$ mutation	
No preceding or allied CML, PV, RARS-T or MDS.	RF grading PVSG, MF Georgii and Thiele
JAK2-MGM clinical staging:	Prefibrotic: RF- 0/1, MF-0, no/minor splenomegaly
Early stage: No anemia with hb and ht in the normal low normal range: hb >13g/dl: early clinical stage	Bone marrow staging:
Intermediate: Hb<13 to >12 g/dl, LDH N or ↑, no leukoerythroblastosis	Early fibrotic ET:RF 2, MF 1, splenomegaly no/minor
Advanced: Hb<10 g/dl, LDH↑↑,	Fibrotic ET: RF3, RCF or MF2, overt splenomegaly
CD34+, leukoerythroblastosis, tear drop	Post-ET MF: RF3/4, or MF-2/3

Table 1: The 2008 European Clinical Molecular and Pathobiological (ECMP) criteria for the diagnosis $JAK2^{V617F}$ 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, IJsselstein, The Netherlands) using FVIII-deficient plasma (Ortho Diagnostic Systems, Beerse, Belgium). Ristocetine (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 × 10^9/L.

Von Willebrand factor (vWF) assays

VWF antigen (VWF: Ag) was assayed by an enzyme-linked immunosorbent assay (ELISA), as described by Cejka [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. Microtiter 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 Na_2HO_4 and 10 µL 30% H_2O_2 was added. After 15 minutes of incubation, the color development was stopped by the addition of 10 µL concentrated acetic acid and the extinction 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 usng 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 electroblotting 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 antiravbbit IgG conjugated to horseradish peroxidase (Bio-Rad, Munich, Germany). Detection of the VWF multimers by luminography was achieved by incubating the nitrocelluse 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 than covered by a translucent polyethylrene 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 $JAK2^{V617F}$ mutated ET including: normal cellular ET, ET with features of early PV (prodromal PV, and ET associated with prefibrotic 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), ht 0.38, MCV 86 fl, platelets 1596 × 10^9/L, leukocytes 9 × 10^9/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, ht 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 × 10^9/L (Figure 1) in the context of hypercellular essential thrombocythemia (Figure 2).

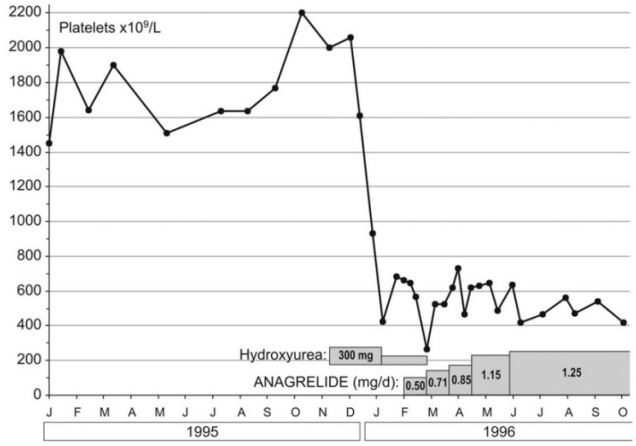

Figure 1: Platelet counts in a 6-year-old boy at time of hemorrhagic thrombocythemia before any treatment and at time of maintained remission of thrombocythemia during short-term treatment with hydroxyurea and subsequent long-term treatment with anagrelide.

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).

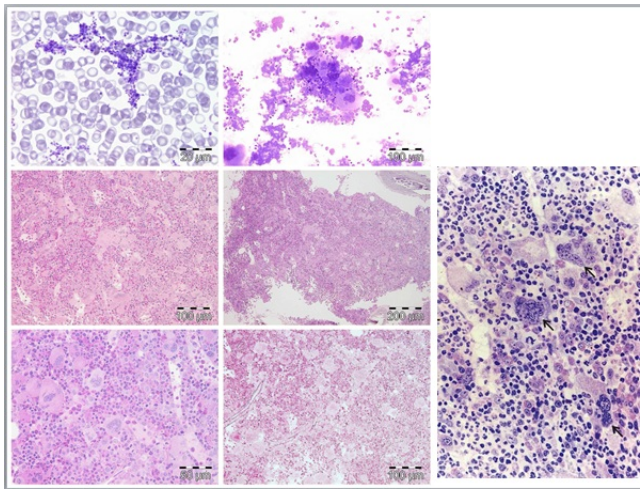

Figure 2: Bone marrow features showing a clearly hypercellular bone with prefibrotic primary megakaryocytic and granulocytic myeloproliferation (PMGM) featured by loose to dense clustering of abnormal megakaryocytes, their variations in size, some naked nuclei and no increase of reticulin fibers (bottom 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 × 10^9/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 × 10^9/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%, segemented 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).

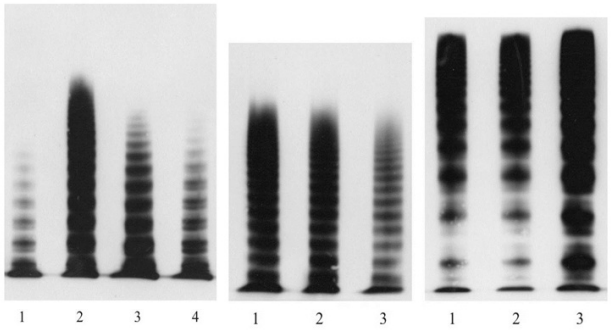

Figure 3: Von Willebrand factor (VWF) multimeric analysis with a low-resolution agarose gel, 1.2% at time of hemorrhagic thrombocythemia and acquired von Willebrand syndrome. Lane 1: plasma from a patient type 2A von Willebrand disease showing the absence of high and intermediate VWF multimers. Lane 2: normal plasma showing the presence of the high, intermediate and low molecular weight VWF multimers. Lane 3: plasma from the 6-year-old boy with hemorrhagic thrombocythemia (platelets 1946 × 10^9/L) showing the absence of the high and some of the intermediate VWF mulimers. Lane 4: plasma from a patient with type 2B von Willebrand disease showing the absence of high and intermediate VWF multimers. Von Willebrand factor (VWF) multimeric analysis with a medium-resolution agarose gel, 1.8%: Lane 1 normal plasma showing a normal pattern of VWF multimers with the presence of high, intermediate and low VWF multimers. Lane 2: plasma from the 6-year-old boy with hemorrhagic thrombocythemia (platelets 1946 × 10^9/L) showing the absence of the high VWF mulimers, an increase of low VWF multimers with prominent fastest subbands of the individual oligimers indicating increased proteolysis of VWF protein. Von Willebrand factor (VWF) multimeric analysis with a medium-resolution agarose gel, 1.8%: lane 1 normal plasma, and lane 2 plasma from normal control showing a normal VWF multimeric pattern and lane 3 thrombocythemia in remission (platelets 450 × 10^9/L) show that all large VWF multimers are present and the triplet structure is normal.

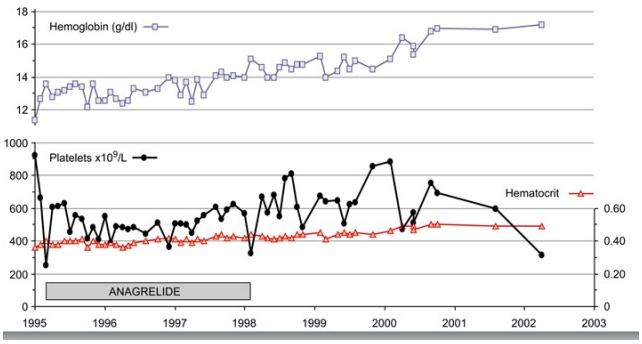

Figure 4: Reappearance of the high molecular-weight VWF multimers.

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 predominant 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 thrombocythemia 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 Thrombocythemia JAK2 and MPL wild type	B1	Primary megakaryotic and granulocytic myeloproliferation PMGM and no or relative reduction of erythyroid 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)
	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 Thrombocytosis, 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	-	-
C1	No leuko-erythroblastose	-	-
	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 PMGM or PMF: Marked increased in reticulin and slight to moderate collagen fibrosis
C2	Splenomegaly	-	-
	Advanced Clinical stage	-	-
	Anemia grade III:Hemaglobin <10g/dl	MF-3	Fibrotic PMGM or PMF: advanced collagen
	Definitive leuko-erythroblastic blood pictures and/or tear drop erythrocytes	-	-
C3	Splenomegaly, Thrombocytopenia, leukocytosis, leukopenia	-	-

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

The karyotype of the nucleated bone marrow cells was found to be normal 46, XY in 36 analysed cells. Molecular studies using southern blot analysis of extracted DNA revealed the absence of a rearrangement within the bcr on chromosome 22. After two years of treatment with anagrelide the dose to control platelet number could be decreased (Figure 4). Anagrelide could be discontinued at the end of 1997 without significant increase of the platelet counts (Figure 4). There was a slight increase of both hemoglobin and hematocrit from low normal to high normal levels during subsequent follow-up of 12 years (Figure 4). During that period up to 2003 the spleen was not palpable. The $JAK2^{V617F}$ mutation was not detectable in 2006. Further studies on peripheral blood, bone marrow and spleen size after 2006 are not available due to loss of follow-up.

Discussion

Hemorrhagic thrombocythemia is defined as a clinical syndrome of spontaneous mucocutaneous hemorrhages associated with extremely high platelet counts in patients with thrombocythemia of various myeloproliferative disorders [22-30]. Several reports in adults demonstrate that hemorrhagic thrombocythaemia 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 thrombocythemia 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-ristocetine 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 thrombocytosis 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 hematopoisesis in $JAK2^{V617F}$ ET is featured by large mature pleomorphic megakaryocytes and normal cellularity of erythropoisesis and myelopoisesis [2,32,33]. The megakaryocytes in polycythemia may have a rather pleiomorphic appearance with a wide range of sizes, including small to giant forms, and the characteristic increase and clustering of larged megakaryocytes. Proliferation of erythropoisesis 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 histopahological feature of PMGM3 according to Georgii et al. [3] is a mixed dual proliferation of increased granulopoesis and megakaryopoesis 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 is almost never seen in $JAK2^{V617F}$ ET or polycythemia vera [32,33].

References

1. MurphyS, Iland H, Rosenthal D, Laszlo J (1986) Essential thrombocythemia. An interim report from the Polycythemia Vera study Group. Sem Hematol 23: 177-182.
2. Michiels JJ, Berneman Z, Schroyens W, Hebeda K, Lam KH, et al. (2015) The 2006 ? 2008 European Clinical, Molecular and Pathobiological)2008 ECMP) Classification and the 2007 WHO revised criteria for myeloproliferative neoplasms. J Hematol Thromb Dis 3: 1.
3. Georgii A, Buhr T, Buesche G, Kreft A, Choritz H (1996) Classification and staging of Ph-negative myeloproliferative disorders by histopathology from bone marrow biopsies. Leukemia Lymphoma 22: 15-39.
4. Michiels JJ, van Genderen PJJ (1997) Essential thrombocythemia in childhood. Sem Thromb Hemostas 23: 295-301.
5. Randi ML, Putti MC, Fabris F, Zanesco L, Girolami A (2000) Features of essential thrombocythaemia in childhood: A study of five children. Brit J Haematol 108: 86-89.
6. Chan GCF, Ma SK, Ha SY, Chan LC, Lau YI (2000) Childhood essential thrombocythaemia without evidence of myeloproliferation. Rit J Haematol 110: 1002.
7. Yang RC, Qian LS (2000) Essential thrombocythaemia in children: A report of nine cases. Brit J Haematol 110: 1009-1010.
8. Thiele J, Kvasnicka H-M, Werden C, Zankovich R, Diehl V, et al. (1996) Idiopathic osteo-myelofibrosis: A clinico-pathological study on 208 patients. Leukemia Lymphoma 22: 303-317.
9. Thiele J, Kvasnicka HM, Boeltken B, Zankovich R, Diehl V, et al. (1999) Initial (prefibrotic stages of idiopathic (primary) myelofibrosis (IMF): a clinicopathlogical study. Leukemia 13: 1741-1748.
10. Thiele J, Kvasnicka HM, Diehl V, Fischer R, Michiels JJ(1999) Clinicopathological diagnosis and differential criteria of thrombocythemias in various myeloproliferative disorders by histopathology, histchemistry and immunostaining from bone marrow biopsies. Leukemia Lymphoma 33: 207-218.
11. Michiels JJ, Thiele J (2002) Clinical and pathological criteria for the diagnosis of essential thrombocythemia, polycythemia vera, and idiopathic myelofibrosis (agnogenic myeloid metaplasia) Int J hematol 76: 133-145.
12. Murphy S, Peterson P, Iland H, Laszlo J (1997) Experience of the Polycythemia vera study group with essential thrombocythemia: A final report on diagnostic criteria, survival, and leukemic transition by treatment. Sem Hematol 34: 29-39.
13. Michiels JJ, Juvonen E (1997) Proposal for revised diagnostic criteria of essential thrombocythemia and polycythemia vera by the Thrombocythemia Vera Study Group. Semin Thromb Hemostas 23: 339-347.
14. Michiels JJ, Raeve DH, Hebeda K, Lam KH, Berneman Z, et al. (2007) WHO bone marrow features and European clinical molecular and pathlogical criteria for the diagnosis and classification of myeloproliferative disorders. Leuk Res 31: 1031-1038.
15. Hayhoe FGJ, Quaglino D (1958) Cytochemical demonstration and measurement of leukocyte alkaline phosphatase activity in normal and pathological states by a modified azo-dye coupling technique Br J Haematol 4: 375-381.
16. ISCN (1978): An International System for Human Cytogenetic Nomenclature. Cytogen Cell genet 21: 309-362.
17. Groffen J, Stephenson JR, Heisterkamp N, De klein A, Bartram CR, et al. (1984) Philadelphia chromosome breakpoints are clustered within or limited to region bcr, on chromosome 22. Cell 36: 93-99.
18. Ivy AC, Nelson D, Bucher G (1941) The standardization of certain factors in the cutaneous "venostasis" bleeding time technique. J Lab Clin Med 84: 160-174.
19. Cejka J (1984) Enzyme immunoassay for FVIII-related antigen. Clin Chem 28: 1356-1358.
20. MacFarlane DF, Stibbe J, Kirby EP (1975) A method for assaying von Willebrand factor (ristocetine cofactor). Thromb Diath Haemorrhag 34: 306-308.
21. Brown JE, Bosak JO (1986) An ELISA test for the binding of von Willebrand factor antigen to collagen. Thromb Res 43: 303-311.
22. Ruggeri ZM, Nilsson IM, Lombardi R, Holmberg L, Zimmerman TS (1982) Aberrant multimeric structure of von Willebrand factor in a new variant of von Willebrand's disease (type IIC). J Clin Invest 70: 1124-1127.
23. Budde U, Schneppenheim R, Plendl M, Dent J, Ruggeri ZM, et al. (1990) Luminographic detection of von Willebrand factor multimers in agarose gels and on nitrocellulose mmbranes Throm Haemostas 63: 312-315.
24. Budde U, Schaefer G, Mueller N, Egli H, Ruggeri ZM, et al. (1984) Acquired von Willebrand disease in the myeloproliferative syndrome. Blood 64: 981-985.

25. Budde U, Dent JA, Berkovitz SD, Ruggeri ZM, Zimmerman TS (1986) Subunit composition of plasma von Willebrand factor in patients with the myleproloferative syndrome. Blood 68: 1213-1217.
26. Budde U, Scharf R, Franke P, Hartmann-Budde K, Dent J, et al. (1993) Elevated platelet count as a cause of abnormal von Willebrand multimer distribution in plasma. Blood 82: 1749-1757.
27. Van Genderen PJJ, Michiels JJ, van der Poel-luitgaarden SCAPM, van Vliet HHDM (1994) Acquired von Willebrand disease as a cause of recurrent mucocutaneous bleeding in primary thrombocythemia: Relationship with platelet count. Ann Hematol 69: 81-84.
28. Van Genderen PJJ, Budde U, Michiels JJ, van Strik R, van Vliet HHDM (1996) The reduction of large von Willebrand factor multimers in plasma in essential thrombocythemia is related to the platelet count. Br J Hematol 93: 962-965.
29. Van Genderen PJJ, Leenknegt H, Michiels JJ (1997) The paradox of bleeding and thrombosis in thrombocythemia: Is von Willebrand factor the link? Semin Thromb Hemostas 23: 385-389.
30. Michiels JJ, Budde U, van der Planken M, van Vliet HHDM, Schroyens W, et al. (2001) Acquired von Willebrand syndromes: Clinical features, aetiology, pathophysilogy, classification and managemen. Baillière"s Clin Haematol 14: 401-436.
31. Michiels JJ. Erythromelalgic Thrombotic Thrombocythemia (ETT) and Hemorrhagic Thrombocythemia (HT) in patients with Essential Thrombocythemia (ET) and Polycythemia Vera (PV. J Hematol Thromb Dis 2: 6.
32. Michiels JJ, Valster F, Wielenga J, Schelfout K, De Raeve H (2015) Euroean vs. World Health Organization clinical molecular and pathological classification of myeloproliferative neoplasms. World Hematol 4: 16-53.
33. Michiels JJ, De Raeve H, Valster F, Potters V, Kim Y, et al. (2017) Extension of 2016 WHO classification into a new set of clinical, laboratory and pathobiological criteria for the diagnosis of myeloproliferative neoplasms: From Dameshek to Vainchenker, Gree and Kralovics. Eur Med J 2: 72-78.
34. Michiels JJ, Prins ME, Hagemeijer A, Brederoo P, va der Meulen J, et al. (1987) Philadelphia-positive essential thrombocythemia and megakryoblast leukemia. Amer J Clin Pathol 88: 645-652.