

## Erythromelalgic Microvascular Disturbances, Major Thrombosis and Hemorrhagic Manifestations of Thrombocythemia in Patients with Essential Thrombocythemia and Polycythemia Vera: Therapeutic Implications

Jan Jacques Michiels\*

Academic Hospital Dijkzigt, Erasmus University Rotterdam (EUR 1974-1998), Department of Hematology, Antwerp University Hospital, Belgium, and Goodheart Institute, Bloodcoagulation and Vascular Medicine Center, Rotterdam, (1998-2014) The Netherlands

### Abstract

Aspirin responsive erythromelalgia is the presenting symptom of thrombocythemia in patients with essential thrombocythemia (ET) and polycythemia vera (PV). Skin punch biopsies taken from the affected areas of erythromelalgia and acrocyanotic complications show typical arteriolar inflammation, fibromuscular intimal proliferation and thrombotic occlusions. If left untreated both microvascular and major thrombosis frequently do occur in thrombocythemia in ET and PV patients, but can easily be cured and prevented by low dose aspirin. The stratification as low, intermediate and high thrombotic risk in the retrospective Bergamo studies has been performed in ET patients not treated with aspirin. The risk of thrombosis in aspirin treated ET and PV is not age dependent when on low dose aspirin, and does recur when not on low dose aspirin during follow-up. The persistence of the Bergamo definition of low, intermediate and high thrombotic risk in the 2012 International Prognostic Score of Thrombosis in ET (IPSET) is applicable for JAK2 mutated ET and PV patients not on aspirin and has led to significant overtreatment with hydroxyurea. MPN disease burden in patients with JAK2<sup>V617F</sup> positive ET and PV is related to JAK2 allele burden and associated leukocytosis, thrombocytosis, constitutional symptoms and splenomegaly. Low dose pegylated interferon (Pegasys<sup>®</sup>, 45 µg/ml once per week or every two weeks) is becoming the first line myeloreductive treatment option to postpone the use of hydroxyurea as long as possible. Von Willebrand factor (VWF) mediated platelet thrombi formation, as well as increased proteolysis of the VWF multimers in one and the same patient do occur simultaneously or in sequence leading to the paradoxical occurrence of thrombosis at platelet count above 400x10<sup>9</sup>/L and bleeding at platelet counts above 1000x10<sup>9</sup>/L due to an acquired von Willebrand disease type 2A.

**Keywords:** Erythromelalgia; Hemorrhages; Arterial thrombosis; Thrombocythemia; Polycythemia vera; Essential thrombocythemia; Hemorrhagic thrombocythemia; Thrombotic thrombocythemia; Aspirin; Myeloproliferative disorders

### Introduction

Mitchel first reported a syndrome of redness and burning pain in the extremities, which were labeled erythromelalgia [1]. The relief of pain for several days after a single dose of aspirin (500 mg) is specific and can be used as a diagnostic criterion for erythromelalgia [2]. We discovered that aspirin responsive erythromelalgia is causally related to thrombocythemia in patients with essential thrombocythemia (ET) and polycythemia vera (PV) at platelet counts around and above 400x10<sup>9</sup>/L [3,4]. By demonstrating that erythromelalgia in thrombocythemia disappeared for 2 to 4 days after a single dose of aspirin (500 mg). This could be attributed to the irreversible inactivation of platelet cyclooxygenase activity for 2 to 4 days by acetyl-salicylic acid (aspirin). As sodium salicylic acid has no cyclo-oxygenase inhibiting activity on platelets and has no effect at all on erythromelalgia we concluded in 1985 that aspirin responsive erythromelalgia in thrombocythemia is caused by platelet mediated microvascular inflammation and thrombosis in ET and PV patients [3-6].

The present study reviews the original description of the broad spectrum of microvascular, thrombotic and hemorrhagic manifestations in ET and PV patients related to peripheral blood cell counts, bone marrow features, and burden of myeloproliferative neoplasm (MPN) disease. The pathophysiology of platelet-mediated arterial thrombosis and bleeding in ET and PV patients is discussed and interpreted in view of the recent literature and updated treatment recommendations for the prevention of platelet-mediated thrombosis and bleeding in thrombocythemia and by reduction of MPN disease burden by pegylated interferon or hydroxyurea.

### Prospective Clinical Studies on Erythromelalgia and Acrocyanotic Toes or Fingers in ET and PV

The broad spectrum of clinical features of erythromelalgia and peripheral, cerebral and coronary ischemic events in 24 patients with myeloproliferative thrombocythemia (11 ET and 13 PVT patients) were collected by the Rotterdam MPN Study Group in the period between 1975 and 1981 (Table 1) [3,4]. Complaints of erythromelalgia consisted of painful red to cyanotic toes or fingers. The pain may vary from prickling, pins and needle stick sensation to a severe burning pain. The involved areas of fore foot toe or fingers are always swollen and painful on palpation and touch (Figures 1 and 2) [3-6]. At time of burning pain severe attacks of tingling prickling may occur [3,4]. The burning pain of erythromelalgia increased on walking compelling the patient to rest with the involved extremity elevated. After rest with the involved extremity elevated, the erythromelalgic redness may disappear but the extremity remains swollen, acrocyanotic and painful on touch. Fingertips or toes remain red, swollen and severely painful like a burn. Acrocyanotic spots in erythromelalgic areas were present in 8 cases indicating localized ischemia (Table 1) [3-6]. In one ET patient

\*Corresponding author: Jan Jacques Michiels, Goodheart Institute, Bloodcoagulation and Vascular Medicine Center, Erasmus Tower, Veenmos 13, 3069 AT Rotterdam, Netherlands, Tel: +316-26970534; Email: [goodheartcenter@upcmail.nl](mailto:goodheartcenter@upcmail.nl)

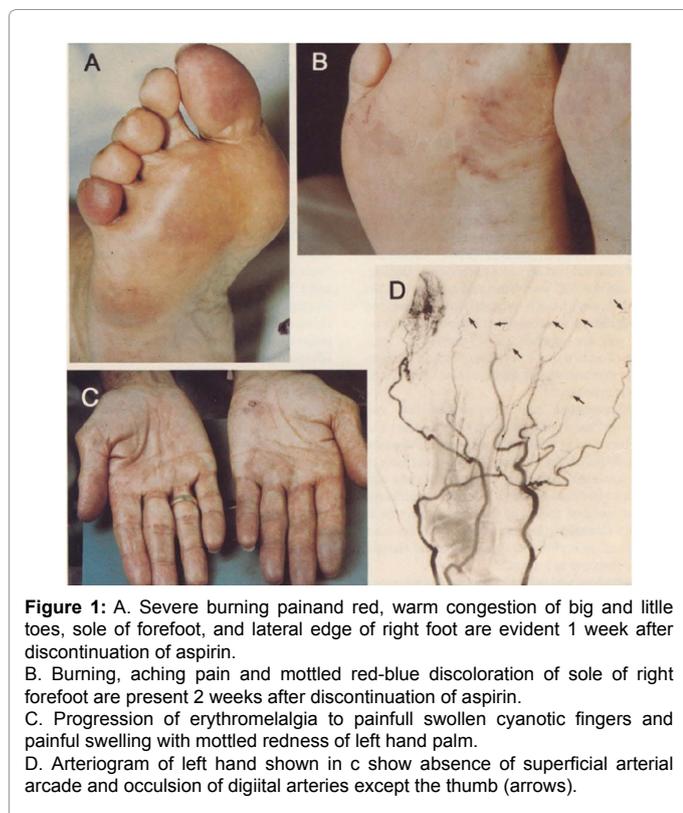
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Number	Gender	Platelets/ 10 <sup>9</sup> /L	Hb/Ht mmol/L %	Erythromelalgia				TIA/ACS	History ETT months
				Toes	Fingers	Skin	Gangrene		
A1 ET	F 62	792	10.3/50	bilateral			present		45
A2 ET	M 39	887	10.0/51	bilateral	-	-	present	TIA/ACS	154
A3 ET	F 42	911	8.9/47	-	unilateral	yes		ACS	60
A4 ET	M 47	614	8.0/39	unilateral	-	-	present	TIA	12
A5 ET	M 46	939	8.3/40	unilateral	-	-	present		4
A6 ET	M 33	742	9.8/45	-	-	-	-	TIA/ACS	20
A7 ET	M 45	567	9.5/46	bilateral		yes		ACS	60
A8 ET	M 39	875	8.8/43	bilateral	bilateral	yes	-	-	30
A9 ET	M 51	690	8.8/45	bilateral	-	-	present	-	20
A10 ET	M 40	1440	8.6/43	unilateral	-	-	present	-	30
A11 ET	F 72	1435	9.4/46	unilateral	-	-	-	-	30
A12 PVT	M 63	1932	11.1/56	bilateral	-	-	-	-	24
A13 PVT	F 75	1800	12.1/62	-	unilateral	yes	-	-	3
A14 PVT	M 61	952	8.3/45	bilateral	-	-	-	-	0
A15 PVT	M 53	636	7.7/39	unilateral	-	-	present	-	36
A16 PVT	F 60	1065	13.4/68	bilateral	-	-	-	-	48
A17 PVT	F 49	728	10.9/57	-	bilateral	-	-	-	1
A18 PVT	M 66	1035	12.2/64	bilateral	-	-	-	-	18
A19 PVT	M 71	1320	13.3/70	unilateral	-	-	-	-	2
A20 PVT	M 65	1300	11.9/65	unilateral	-	-	present	-	24
A21 PVT	F 55	1085	12.2/61	-	unilateral	-	-	-	4
A22 PVT	F 59	708	11.0/59	-	bilateral	yes	-	-	3
A23 PVT	F 74	959	13.1/72	unilateral	unilateral	-	-	-	24
A24 PVT	M 71	609	12.5/66		unilateral				6

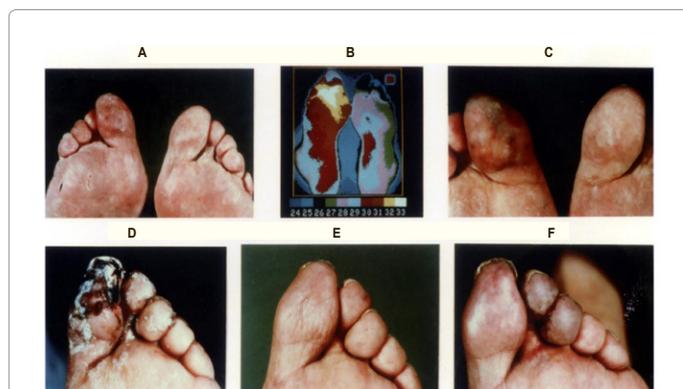
Table 1: Erythromelalgia and microvascular disturbances in 24 patients with ETT (11 ET and 13 PV) [3].



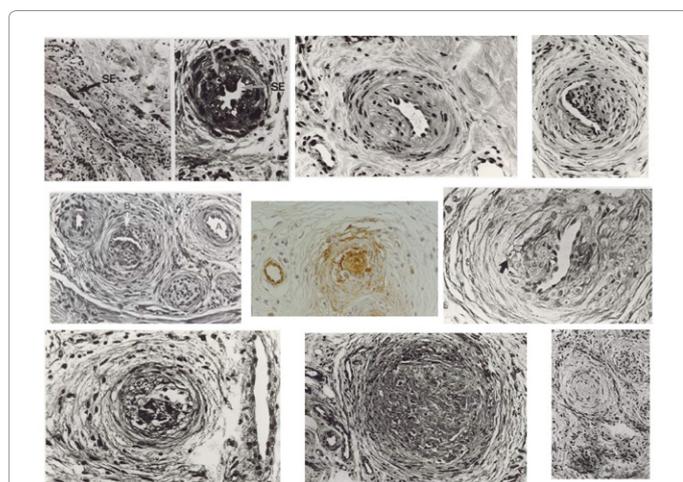
**Figure 1:** A. Severe burning pain and red, warm congestion of big and little toes, sole of forefoot, and lateral edge of right foot are evident 1 week after discontinuation of aspirin. B. Burning, aching pain and mottled red-blue discoloration of sole of right forefoot are present 2 weeks after discontinuation of aspirin. C. Progression of erythromelalgia to painful swollen cyanotic fingers and painful swelling with mottled redness of left hand palm. D. Arteriogram of left hand shown in c show absence of superficial arterial arcade and occlusion of digital arteries except the thumb (arrows).

(A11), warm painful swollen redness of the big toe was accompanied by a cold dark blue cyanotic non-swollen third toe. In a third patient (A24, PV) who presented with typical pronounced erythromelalgia of

the fingertips was followed by cold, swollen acrocyanosis of 4 fingers of the left hand (aspirin responsive blue finger and black toe syndrome in PVT, Figure 1) [1-4]. In ET erythromelalgia already develops at platelet count in excess of 350 to 400x10<sup>9</sup>/L. When symptoms occur at 'normal' platelet count around 350x10<sup>9</sup>/L in PV in remission by phlebotomy alone platelet count usually rise to values above 400x10<sup>9</sup>/L during aspirin treatment [3,4]. The time lapse between first complaints of typical erythromelalgia and the diagnosis of ET or PV elapsed from a few months to 5 years, (mean 2,25) years [3,4]. One ET patient (A2) had a very long history of erythromelalgia complicated by peripheral gangrene, atypical TIAs and recurrent coronary ischemic events. Seven cases with longstanding erythromelalgic signs and symptoms developed ischemic complications: acrocyanosis with blister formation of the big toe (A1), a necrotic spot in the tip of a toe in 4 (A1, A1, A4, A20) [3,4]. In PV case 20 with red congestion of the big and second toe and a necrotic spot of the third toe bone marrow biopsy from the anterior iliac crest was complicated by wound dehiscence and subcutaneous ecchymosis. Sympathectomy because of ischemic complication was not effective in 5 cases with blue black toes and digital gangrene (thromboangiitis obliterans) in the absence of infection and inflammation in 3 (A4, A5, A10), and necrosis of the distal phalanx of a toe in 2 (A2, A9). Angiogram in one ET case (A1) with erythromelalgia complicated by digital gangrene showed abrupt occlusions of toe arteries without any evidence of vascular pathology of the supplying arteries [5,6]. The sequential occurrence of aspirin responsive erythromelalgic, ocular and cerebral microvascular disturbances in relation to platelet counts and the effects of platelet reduction during 15 years follow-up has been described in detail 6. Transient cerebral ischemic events in one PV patient (A24) were followed by overt erythromelalgia in the finger tips, which were left untreated and complicated by acrocyanosis of 4 fingers of the left hand showing abrupt arterial occlusions of the hand arcade and of supplying digital finger arteries of the 4 involved acrocyanotic swollen



**Figure 2:** A. burning pain and red congestion of forefoot sole and second and third toes and bluish discoloration of very painful big toe of right foot (see also C). B. Thermogram of right foot showing surface skin temperature with erythromelalgia exceeding 310 Celsius (see also A and C). C. Detail of very painful and bluish ischemic big toe with skin surface temperature 50 Celsius lower compared to erythromelalgic forefoot sole in A and B. D. Progression of untreated erythromelalgia into painful "black" big toe and peeling of the skin of second and third toes while on coumarin treatment. E. Complete restoration of painful ischemic big toe and erythromelalgic second and third toe within one week by treatment with aspirin 500 mg once daily. F. Relapse of painful red blue toes did occur 2 weeks after discontinuation of aspirin by the patient. After reinstatement the erythromelalgia disappeared again, which was interpreted by the patient as "The Miracle of Rotterdam"



**Figure 3:** Histology of erythromelalgia one week after discontinuation of aspirin showing arterioles with pronounced fibromuscular intima proliferation with swollen endothelial cells and muscle cell (Figure 4) proliferation of the inner part of media with associated degenerative vacuola cytoplasmic swelling (upper panels and left middle). Fragmented internal elastic lamina (arrow) between proliferated smooth muscle cells in the van Gieson stain (right middle panel): fibromuscular intimal proliferation. Histology of erythromelalgia complicated by acrocyanotic ischemia 3 weeks after discontinuation of aspirin in essential thrombocythemia showing partial thrombotic occlusion of arteriole with fibromuscular intimal proliferation (lower left). Occlusion of arteriole by organized thrombus with perivascular fibrosis (lower middle). Endstage vascular pathology of acrocyanotic erythromelalgia showing onion-like appearance of fibrosed occluded arteriole. Immunofluorescence of a biopsy from an erythromelalgic area with an arteriole occluded by a platelet rich thrombus with a strong staining for von Willebrand factor of the thrombus and of endothelial cells (central panel) [7].

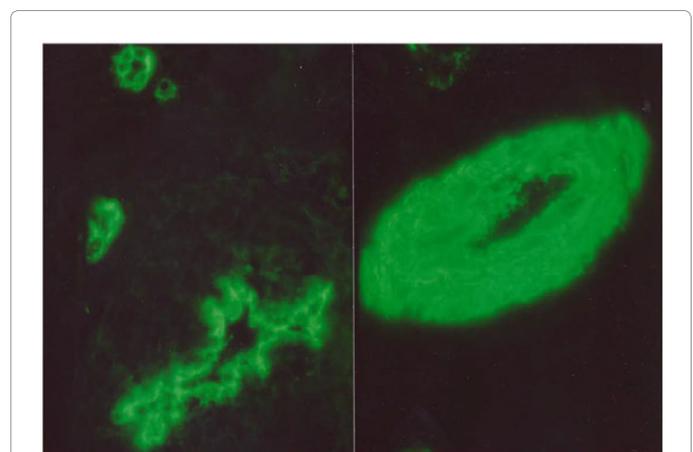
fingers (Figure 1) [5,6]. Strikingly, the arterial pulses of arteriae dorsales pedis and arteriae tibiales remain normal in all cases of erythromelalgia complicated by peripheral acrocyanosis or digital gangrene. Complete

relief of erythromelalgic and/or ischemic pain and restoration of the ischemic acral circulation disturbances (thrombo-angiitis obliterans) in ET and PVT patients could be obtained by long-term treatment with low-dose aspirin (250 mg/day). In the five patients with erythromelalgia complicated by digital gangrene (Table 1) [3,4] experienced the favorable effect of aspirin, but it took about one or two weeks before all ischemic symptoms completely disappeared [5,6]. If left untreated, cold cyanotic fingertip or toe tips may develop, remain painful and resistant to aspirin very likely due to irreversible digital arteriolar occlusion. Standard therapy with oral anticoagulants (warfarin) and reduction of hematocrit to normal by bloodletting in PVT did not affect the erythromelalgic ischemic symptoms and acrocyanotic digital ischemia and gangrene (thrombo-angiitis obliterans).

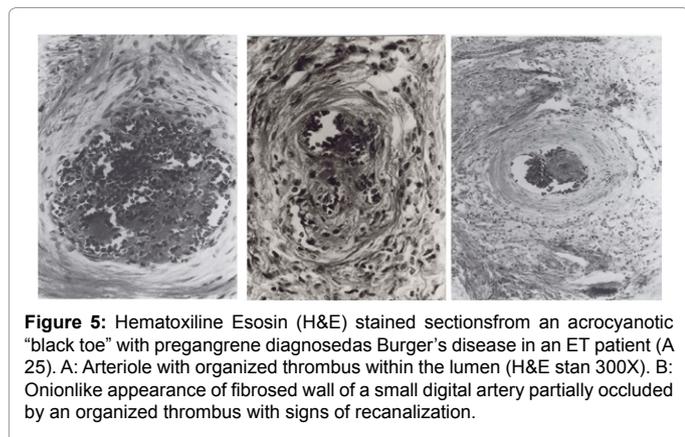
### Histopathology of Erythromelalgia in Thrombocythemia

In the 1970s very little was known about the histopathology of erythromelalgia in thrombocythemia as the opportunity to examine tissue from the involved extremities of typical fresh erythromelalgia and of its acrocyanotic complications was not or only scarcely available [7]. We performed between 1975 and 1980 skin punch biopsies from erythromelalgic areas and from acrocyanotic areas in thrombocythemia patients (ET and PV) [7]. The histological appearances of a skin punch biopsy from an area of recently relapsed erythromelalgia 1 week after discontinuation of aspirin, show arteriolar lesions without involvement of venules, capillaries of nerves (Figure 3) [7]. Arterioles showed pronounced proliferation and degeneration in their vessel walls. The lining endothelial cells are swollen some with large nuclei. The inner layer of the arteriolar vessel wall was thickened by intimal proliferation of cells with narrowing of the lumen. With factor VIII antisera an affected arteriole with intimal thickening shows an inner layer of fluorescent endothelial cells (Figure 4) and with the antiserum against smooth muscle cells strong fluorescence was seen in the proliferated subendothelial intimal cell zone (fibromuscular intimal proliferation, Figure 3) [7]. Slight intravascular and perivascular infiltration with inflammatory cells and some perivascular fibrosis is present. The internal elastic lamina is seen to be splitted by proliferating smooth muscle cells (fibromuscular intimal proliferation, Figure 3) [7].

The histopathological lesions from relapsed erythromelalgia complicated by bluish spots 3 weeks after discontinuation of aspirin, show arteriolar lesions of fibromuscular intimal proliferation



**Figure 4:** Immunofluorescence of platelet-mediated arteriolar intima thickening from erythromelalgic area in essential thrombocythemia showing a single layer of endothelial cells with factor VIII antiserum (left) and of proliferated muscle cells with smooth muscle antiserum in an injured arteriole (right).



**Figure 5:** Hematoxiline Eosin (H&E) stained sections from an acrocyanotic "black toe" with pregangrene diagnosed as Burger's disease in an ET patient (A 25). A: Arteriole with organized thrombus within the lumen (H&E stain 300X). B: Onionlike appearance of fibrosed wall of a small digital artery partially occluded by an organized thrombus with signs of recanalization.

occluded by an organized fresh von willebrand factor rich platelet thrombi (Figure 3). The skin wedge excision from an acrocyanotic erythromelalgic toe mainly shows arterioles with fibromuscular intimal proliferation with narrowing of the lumen and on top of that occluded arterioles by fresh organized thrombus is seen in erythromelalgic areas complicated by acrocyanosis (Figures 3 and 5). The thickened arteriolar microvasculature ultimately show occluded arterioles by thrombi with perivascular fibrosis without inflammatory cell infiltration give rise to onion-like structures, in which the internal lamina elastic appears to be absent and the external elastic membrane is fragmented (Figure 3) [7].

### Erythromelalgic Cerebral and Ocular Ischemic Manifestations in ET and PV

In the original cohort of 24 patients with myeloproliferative thrombocythemia (11 ET, 13 PVT) aspirin responsive migraine-like atypical transient ischemic attacks (MIA) and acute coronary syndrome (ACS) were observed in eight patients (5 ET, 3 PVT, Table 1) [3,4]. Recurrent sudden attacks of unconsciousness lasting for a few minutes did occur in 3 patients with erythromelalgia (A2, A3, A16). Migraine like headache followed by recurrent transient hemiparesis lasting for half an hour (migraine accompagnée) was associated with disorientation in time [8] in one patient with a history of myocardial infarction. One case suffered from severe migraine-like headache followed by nausea and emesis after discontinuation of aspirin (A7). On case with a history of myocardial infarction suffered from recurrent transient ischemic attacks (A6). In 1993 we reported on the presenting transient neurologic and ocular manifestation in ET patients [9] and subsequently reviewed the literature [10,11]. The transient neurologic and ocular symptoms in ET are featured by migraine like atypical transient cerebral or ocular ischemic attacks (MIAs) [9-11]. The main neurologic symptoms in ET are poorly localized cerebral symptoms of unsteadiness, unstable gait, or attacks of sudden falling without loss of consciousness or weakness, focal transient cerebral symptoms of mono- or hemiparesis and focal transient visual symptoms of monocular blindness [9]. The cerebral or visual symptoms are accompanied by migraine-like dull or throbbing unilateral or bilateral headaches, usually lasting for several hours. None of the patients had a history of migraine. Global visual symptoms include scintillating scotomas and blurred vision. All transient neurologic and visual attacks had a sudden onset and occurred gradually rather than all at once and lasted for a few seconds to several minutes. The progression had both a rapid (within seconds) spreading of visual symptoms and a sequential occurrence of one symptom after another; for instance migraine-like visual symptoms followed by dysarthria, followed by weakness [9]. Progression of transient hemiparesis to stroke or permanent blindness did occur if not treated with aspirin. Routine

screening with brain CT, carotid duplex, ultrasonography, or cardiac investigations did not reveal any obvious coexistent abnormality of cause of MIAs in affected ET and PV patients [9-11].

Three cases (1ET and 2 PV) developed necrotic toes and were diagnosed as thrombo-angiitis obliterans (Buerger's disease) of the toes, which were already amputated by a surgeon before referral to us because of thrombocythemia for expert evaluation and treatment recommendation.

### The 1980 Rotterdam Clinical and Pathological Criteria for ET and PV

Focusing between 1975 and 1980 on the causal relation between erythromelalgia and thrombocythemia in ET and PV patients, we were able to document the very early stage of ET by the use of the Rotterdam Clinical and Pathological (RCP) criteria for ET and PV (Table 2) [3,4]. Since 1975 we found platelets in excess of  $400 \times 10^9/L$ , and an increase of clustered enlarged megakaryocytes in a bone marrow biopsy material diagnostic for ET (Table 2) [3,4]. Iron stain of bone marrow histology specimens from ET and PV patients were performed with Prussian blue reagents. The RCP criteria of ET and PV were determined in 1980 based on careful prospective documentation of peripheral blood and bone marrow histology from bone marrow biopsy material. Platelets in excess of  $400 \times 10^9/L$ , and an increase of clustered enlarged megakaryocytes in a bone marrow biopsy material was found to be diagnostic for ET and excluded reactive thrombocytosis (Table 2). The minimum criterion for the diagnosis according to the PVSG in 1975 was  $1000 \times 10^9/L$  [12], which was reduced to  $600 \times 10^9/L$  in 1986 [13,14].

Between 1975 and 1980 we used bone marrow histology as the

S. No	Criteria
A	Major criteria
	A1 Persistent platelet count in excess of $400 \times 10^9/L$ . A2 Increase and clustering of large megakaryocytes in bone marrow biopsy
B	Confirmative criteria
	B1 Presence of large platelets in a peripheral blood smear
	B2 Absence of any underlying disease for reactive thrombocytosis and normal ESR.
	B3 No or slight splenomegaly on palpation or scan (<15 cm) B4 Increase of LAP-score and no signs of fever or inflammation
C	Exclusion criterion
	Ph+ chromosome and any other cytogenetic abnormality in blood or bone marrow cells
	B. The 1980 RCP major (A) and minor (B) criteria for prefibrotic PV3
	A1 Raised red cell mass. Male >36 ml/kg, female >32 ml/kg: PVSG 1975 <sup>14</sup>
	A2 Increased erythrocyte count above $6 \times 10^{12}/L$ .
	A3 Increase in bone marrow biopsy of clustered, large pleomorphic megakaryocytes with hyperlobulated nuclei and increased cellularity due to increased megakaryopoiesis erythropoiesis or typically trilinear mega-erythro-granulopoiesis. typical PV bone marrow excludes erythrocytosis <sup>9,18,19</sup> .
	B1 Thrombocythemia, persistent increase of platelet $>400 \times 10^9/L$
	B2 Leukocytosis, leucocyte count $>10^9/L$ and low erythrocyte sedimentation rate
	B3 Raised leukocyte alkaline phosphatase (LAP) score $>100$ , absence of infection
	B4 Splenomegaly on palpation or on isotope/ultrasound scanning
A1 or A2 plus A3 and none of B establishes erythrocythemic PV	
A1 or A2 plus A3 plus one of B establishes PV and excludes erythrocytosis	

**Table 2:** The Rotterdam Clinical and pathological (1980 RCP) criteria of essential thrombocythemia (ET) defined and used between 1975-1980 [3].

Number	Diagnosis	Gender	Spleen	Spleen	Platelets	Hb	Ht	Erythrocyt	BM iron
		Age yrs		scan cm	10 <sup>9</sup> /L	mmol/l		10 <sup>12</sup> /L	stain
A1	ET	F 60	np	10.6	792	10.4	0.49	6.7	neg
A2	ET	M 39	np	9.8	887	10	0.51	6	pos
A3	ET	F 42	-	5	911	8.9	0.47	5.4	pos
A4	ET	M 47	p	13.5	614	8	0.39	4.5	-
A5	ET	M 46	np	10.5	939	8.3	0.4	4.4	pos
A6	ET	M33	p	14.3	742	9.8	0.49	5.8	pos
A7	ET	M 45	np	11.2	567	9.5	0.46	5.2	pos
A8	ET	M39	np	10.8	875	8.8	0.43	4.9	pos
A9	ET	M51	np	11.2	690	8.8	0.45	5.5	pos
A10	ET	M40	p	14.4	1440	8.6	0.43	4.7	pos
A11	ET	F 72	p	14.8	1435	9.4	0.46	6.1	neg
A12	PTV	M 63	np	8	1932	11.1	0.56	6.5	neg
A13	PTV	F 75	np	-	1800	12.1	0.62	7.6	neg
A14	PTVb	M 61	-	5	952	8.3	0.45	5.6	neg
A15	PTVb	M 53	p	13.1	636	7.7	0.39	5.4	neg
A16	PTV	F 60	p	14.5	1065	13.4	0.68	7.9	neg
A17	PTV	F 49	p	15.8	728	10.9	0.57	7.5	neg
A18	PTV	M 66	p	15.9	1035	12.2	0.64	7.1	neg
A19	PTV	M 71	p	15.3	1320	13.3	0.7	6.4	neg
A20	PTV	M 65	p	14.9	1300	11.9	0.65	7.6	neg
A21	PTV	F 55	p	14.1	1085	12.1	0.61	7.1	neg
A22	PTV	F 59	p	12.5	708	11	0.59	7.5	neg
A23	PTV	F 74	p	14.4	959	13.1	0.72	9.1	neg
A24	PTV	M 71	p	16.6	609	12.5	0.66	9.9	neg
B1	PV/HT	M 50	p	-	2316	10.2	0.52	6.3	neg
B2	PV/HT	F 86	p	19	2975	5.3	0.32	4.4	neg
B3	ET/HT	M 72	np	11.4	1503	6.3	0.31	3.5	pos
B4	ET/HT	F 45	np	13.8	1074	8.1	0.41	4.6	neg
B5	ET/HT	M 51	np	10.1	1080	8.3	0.42	4.9	-
B6	PV/HT	M 61	np	11.4	737	9.9	0.49	5.1	neg

**Table 3a:** Clinical and peripheral blood data in 14 ET and 16 PTV patients (Source Michiels Thesis 1981[1];A1/24 complicated by erythromelalgia and B1/6 hemorrhagic thrombocythemia (HT) [3]. ET: Essential Thrombocythemia, PTV: Polycythemia Thrombocythemia Vera. b= Treated with Busulphan, np: Not Palpable, p=Palpable Source. Michiels J.J. Thesis, Erythromelalgia and Thrombocythemia, Rotterdam 198:160-163.

pathognomonic clue to PV on top of the 1975 PVSG criteria for the diagnosis of polycythemia vera [14,15]. Erythrocyte volume (red cell mass RCM) was measured using Cr51 (natriumchromate) labeled autologous erythrocyte1. The RCP modifications in 1980 of the 1975 PVSG criteria for PV14 include 4 main changes (Table 2) [3,4]. First, the major criterion O2-saturation of >92% is deleted since a bone biopsy differentiates between PV and erythrocytosis9. Second; splenomegaly is replaced by bone marrow histology as a major criterion (A3, Table 2). Third, the RCP diagnostic set used splenomegaly as a minor criterion (Table 3a). Fourth, we skipped raised B12 (>900 ng/L) or raised B12 binding capacity (>2200 ng/L) as completely irrelevant for the diagnosis of early and overt stage PV (Table 2).

### Prospective Laboratory Investigations and Bone Marrow Features of Thrombocythemia in ET and PV

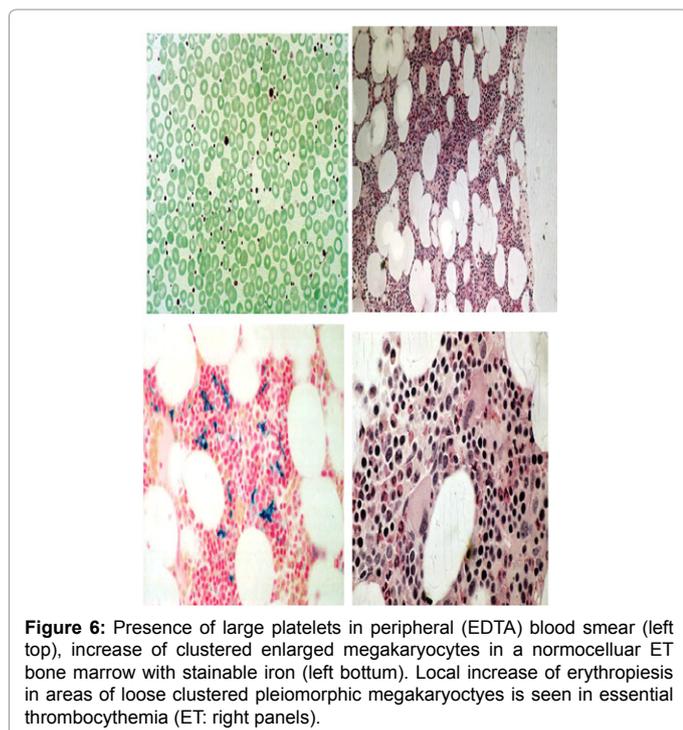
Platelet-mediated arteriolar inflammation and platelet thrombi (erythromelalgia) did not occur in reactive thrombocytosis, which strongly indicates not only a quantitative but also a qualitative disorder of platelet hyperfunction in thrombocythemia [3,4,8,10]. This unknown platelet defect in thrombocythemia in ET and PV patients is postulated by Michiels and co-workers to lead to an increased risk of microvascular thrombotic complication and microvascular and macrovascular thrombotic complication in thrombocythemia of various MPNs [16-20]. Phlebotomy alone in PV significantly reduced the incidence of

macrovascular complication at normal hematocrits of 0.40 or less. A hematocrit of <0.45 is not low enough to prevent major thrombosis. The microvascular syndrome associated thrombocythemia persist in PV in hematological remission by phlebotomy alone. Platelet mediated microvascular thrombotic disease in ET and PV is best controlled by low dose aspirin (100 mg/day) or selective reduction of platelet counts to normal, (<350x10<sup>9</sup>/L). We have elucidated the pathophysiology of aspirin sensitive platelet thrombophilia in ET and PV patients in several original basic research publications and in one recent insight review articles [16-20].

In the period of January 1975 to December 31, 1980, we prospectively studied the laboratory and bone marrow features in 30 consecutive early prefibrotic stage MPN patients, who presented with erythromelalgic thrombotic thrombocythemia (ETT), 14 ET and 16 PV patients (Table 3b) [3,4]. The clinical features of erythromelalgia caused by platelet-mediated arteriolar inflammation and thrombosis in thrombocythemia of the ET and PV cases have been reported in great detail in the *Annals of Internal Medicine*, 19854. The mean age of 30 ETT patients (ET and PV) was 56.7 (range 33-96) years. Eleven of 14 ET patients had platelet counts below 1000x10<sup>9</sup>/L, in which the PVSG diagnosis of ET (Table 2) would have been overlooked by the PVSG criteria at that time. Spleen size on scan was slightly increased in 5 of 14 ET and in 13 of 16 PV patients. Leukocyte count counts was increased (>10x10<sup>9</sup>/L) in 5 out of 14 patients with ET and in 14 of 16

Number	Leukoc	LAF	RCM Ery	ESR	BM	BM1	BM1	BM M/E	BM iron
	10 <sup>9</sup> /L	score	volume		megakar	cellularity	reticuline	ratio	stain
A1 ET	10	183	31	2	1+	N	N	2.1	neg
A2 ET	9	155	-	0	1+	N	N	2.9	pos
A3 ET	8	109	-	1	2+	2+	1+	3.3	pos
A4 ET	9	101	-	4	-	-	-	-	-
A5 ET	16	128	26	2	1+	N	N	3	pos
A6 ET	7	139	-	6	1+	N	N	3.1	pos
A7 ET	8	127	-	3	1+	N	N	2.9	pos
A8 ET	10	38	-	3	2+	1+	1+	-	pos
A9 ET	10	103	-	52	1+	1+	1+	3.2	pos
A10 ET	11	60	-	5	1+	N	N	4	pos
A11 ET	13	113	32	3	2+	1+	2+	1.9	neg
A12 PV	10	207	59	75	1+	2+	1+	1.1	neg
A13 PV	28	193	-	2	2+	2+	N	1.7	neg
A14 PV	13	236	-	4	2+	2+	2+	1.1	neg
A15 PV	11	103	-	63	2+	1+	1+	1.6	neg
A16 PV	17	243	45	3	2+	2+	1+	1.2	neg
A17 PV	8	186	60	1	1+	1+	1+	0.6	neg
A18 PV	14	184	63	2	2+	1+	1+	2.2	neg
A19 PV	16	219	50	1	2+	2+	1+	3.3	neg
A20 PV	18	128	38	3	2+	1+	1+	1.2	neg
A21 PV	13	170	43	1	2+	2+	2+	2.1	neg
A22 PV	17	168	42	0	2+	2+	2+	1.3	neg
A23 PV	9	219	54	2	2+	2+	1+	0.5	neg
A24 PV	18	215	82	0	2+	2+	1+	1.3	neg
B1 HT	46	-	-	0	2+	2+	2+	-	neg
B2 HT	24	235	38	1	2+	2+	2+	0.6	neg
B3 HT	8	49	-	4	2+	N	1+	2.2	pos
B4 HT	7	105	-	7	1+	N	N	2.5	neg
B5 HT	10	47	-	2	2+	2+	2+	-	-
B6 HT	8	121	-	36	1+	N	1+	1.8	neg

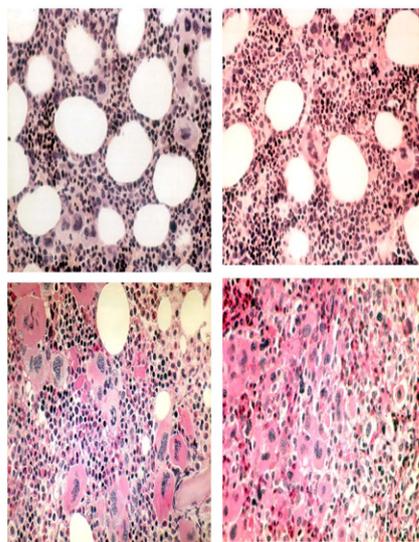
**Table 3b:** Peripheral blood and bone marrow data in 14 ET and 16 PV patients (Source Michiels Thesis 1981 [1]): A1/24 complicated by erythromelalgia, and B1/6 hemorrhagic thrombocythemia (HT) [3]. Source. Michiels J.J. Thesis, Erythromelalgia and Thrombocythemia, Rotterdam 1981:160-163.



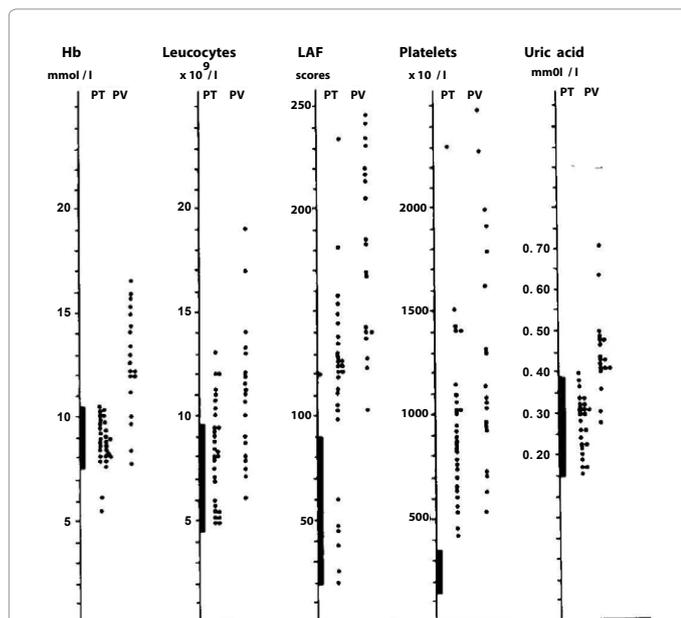
**Figure 6:** Presence of large platelets in peripheral (EDTA) blood smear (left top), increase of clustered enlarged megakaryocytes in a normocellular ET bone marrow with stainable iron (left bottom). Local increase of erythropoiesis in areas of loose clustered pleiomorphic megakaryocytes is seen in essential thrombocythemia (ET: right panels).

PV patients. LAP score was increased (>100) in 12 out of 14 ET and in all PV patients.

Increase of clustered large megakaryocytes and a normoblastic erythropoiesis (decreased M/E ratio) was present in bone marrow smears and biopsies of 14 ET and 16 PV patients (Table 3b, Figures 6 and 7). The myeloid/erythrocyte (M/E) ratio was shifted towards increased erythropoiesis in half of ET and most PV patients. The mean M/E ratio in 14 patients with early stage ET was 1.95 (range 0.6-4.0) as compared to 1.53 (range 0.6-3.3) in 16 PV patients. A normocellular bone marrow picture with increase of clustered pleiomorphic megakaryocytes and no increase of cellularity (Figure 5) was seen in 7 of 14 ET and in one of 16 PV patients. A moderate increase of cellularity (1+=60-80%) in the bone marrow due to increased erythropoiesis (=decreased M/E ratio) leading to a ET/PV picture was seen in 3 ET and 4 PV patients (Figure 7). A typical PV bone marrow picture with pronounced increase of cellularity (2+=80-100%) due to predominantly increased erythropoiesis or trilinear hypercellular hematopoiesis (megakaryo/erythro/granulocytic: panmyelosis Figure 6) was seen in 2 of 14 ET patients and in 11 of 16 PV. These results indicate that bone marrow histopathology on its own is not reliable to clearly differentiate between ET and PV but appeared to be a powerful tool to differentiate ET and PV from all variants of primary or secondary erythrocytosis and reactive thrombocythosis with a sensitivity and specificity near to 100% [3,4]. The morphology of large pleiomorphic megakaryocytes were not different in ET and PV except that pleiomorphism of megakaryocytes



**Figure 7:** ET (left upper), PV (right upper, left bottom), and trilineear PV (right bottom) bone marrow histology seen in ET and PV patients. Pleiomorphic megakaryocytes in ET (upper panels) have similar hyperlobulated nuclei as in PV (bottom panels). Dense clustered pleiomorphic megakaryocytes (PV/RF 2, right bottom) is seen in advanced PV show dysmorphic nuclei but not cloud-like. Please note relative increased erythropiesis in ET (upper panels).



**Figure 8:** Laboratory findings at time of presentation in symptomatic with thrombocythemia subdivided in 30 PT (=ET) and 20 PV patients seen between 1975 and 1985. Hb levels are normal in PT and elevated in PV 16. Leucocytes were normal or elevated in PT and PV. LAF score was much more elevated in PV than in PT with normal values in 6 PT patients. Platelet counts were between 400 and 1000x10<sup>9</sup>/L in the majority of the 30 PT patients. Serum uric acid levels were normal in PT and usually elevated in PV.

was more pronounced in the hypercellular (80-100%) bone marrows of PV and ET patients with advanced myeloproliferative disease (Figure 7) [3,4]. We could conclude in 19813 that RCP defined ET and PV patients show overlapping bone marrow pictures with increase of pleomorphic clustered megakaryocytes and varying degrees of increased erythropiesis at time of diagnosis (Figures 6 and 7).

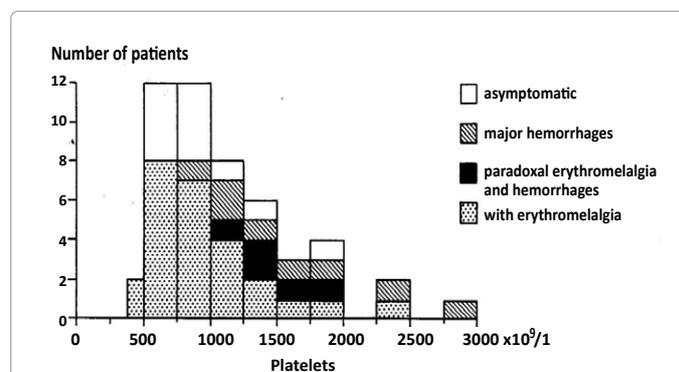
## Erythromelalgic Thrombotic Thrombocythemia (EET) and Hemorrhagic Thrombocythemia (HT)

In the period we reported the erythromelalgic, thrombotic and hemorrhagic manifestations in 50 consecutive cases of thrombocythemia in 30 ET and 20 PV patients 10: 16 ET and 15 PV patients suffered from erythromelalgic thrombotic thrombocythemia (ETT). Besides erythromelalgia 16 thrombocythemia patients suffered from additional transient arterial ischemic or thrombotic complications involving the brain or heart : transient cerebral neurologic ischemic attacks (TIA) in 3, and migraine-like atypical TIAs in 8 ; myocardial infarction in 2; amourosis fugax in 2 and venous thrombosis was not observed. Hemorrhagic thrombocythemia (HT) was diagnosed in 8 patients without erythromelalgia. The presenting features of HT were bruises and ecchymoses in 4, recurrent epistaxis in 2 bleeding after tooth extraction in 2 and gastrointestinal bleeding in 2. Paradoxical occurrence of erythromelalgia and bleeding symptoms (ETTT plus HT) was observed in 5 thrombocythemia patients. The laboratory data at time of presentation in 30 ET and 20 PV patients are shown in Figure 8. Hemoglobin levels were normal in ET and elevated in PV. Leukocyte counts were normal or increased in ET and PV. The leukocyte alkaline phosphatase score was much more elevated in PV than in ET (Figure 8).

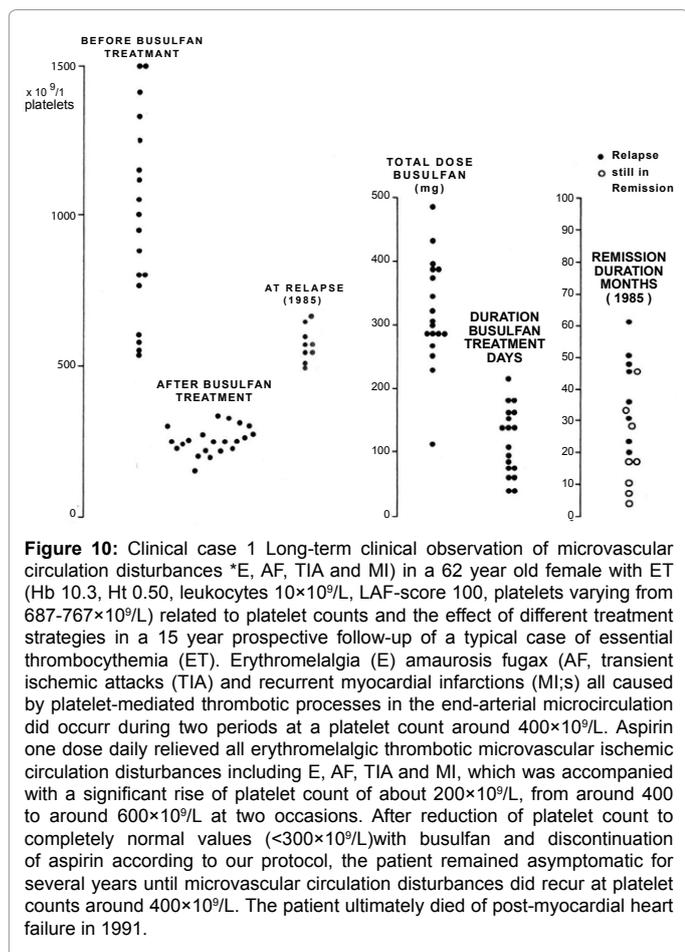
Seventeen of 31 patients with erythromelalgic thrombotic thrombocythemia had platelet counts below 1000x10<sup>9</sup>/L with the lowest count of 420x10<sup>9</sup>/L (Figure 8) [10]. Platelet counts in 8 HT patients and 5 ETT/HT patients with paradoxal erythromelalgia and bleeding had platelet counts between 850 to 3000x10<sup>9</sup>/L and were in excess of 1000x10<sup>9</sup>/L in 12 of 13 thrombocythemia patients (Figure 9). Budde et al discovered that increased platelet count far above 1000x10<sup>9</sup>/L in HT was associated with an acquired von Willebrand syndrome type 2A [21,22]. Michiels and co-workers prospectively documented in ET and PV patients the details of paradoxal occurrences of erythromelalgic microvascular thrombotic manifestation and spontaneous mucocutaneous bleeding at platelet count above 1000x10<sup>9</sup>/L during long-term follow-up caused by an acquired von Willebrand syndrome type 2A, which disappeared after correction of platelet count to normal (Figures 10-12) [20,23-27].

## Platelet Lowering Agents in ET: Low Dose Busulphan to Control Platelet Number and ETT Symptoms

The European Organization on Research and Treatment of Cancer (EORTC) conducted phase III study in previously untreated PV



**Figure 9:** Frequency distribution of platelet counts in 50 patients (30 ET and 20 PV) associated with erythromelalgic thrombotic thrombocythemia (ETT, N=31), hemorrhagic thrombocythemia (HT, N=8) and paradoxical occurrence of ETT and HT (N=5) Michiels et al.



patients \*n=293). Previously untreated PV patients were randomized for P32 and busulfan in the years 1967-1978 [28]. The hematologic objective response criteria were given in terms of hematocrit values between 0.42 and 0.47. With a median follow-up of 9 years, busulfan treatment (mean dose 310 mg) was better than P32 (mean dose 5.9 mCi) [28]. There were fewer thrombotic episodes with busulfan than with P32 due to better control of the thrombocythemia with busulfan: 25 of 149 treated with P32 died of vascular complication compared to 8 of 145 treated with busulfan, but the incidence of acute leukemia after a median follow-up of 9 years was equally low and not statistically different between the two agents (2/140 P32 versus 3/145 busulphan [28]. Overall survival at 10 years was significantly better with busulphan (74%) than with P32 (55%). This difference in overall survival was due to a much higher frequency of vascular accidents in the P32 arm because of a poorer control of hemoglobin values and platelet counts. Consequently, twenty five of 149 patients treated by P32 died from vascular complications not on aspirin compared to 8 of 145 PV patients treated by busulphan not on aspirin [28].

The London Myeloproliferative Study Group of Wetherley-Mein et al used the alkylating agent busulphan more judiciously as an effective and safe platelet reductive agent for the control of thrombocythemia in PV [29], and for the control of platelet count in symptomatic cases of ET30. In the London ET study, Wetherley-Mein and co-workers reported on the effective treatment with busulphan in 37 cases (mean age 60.5, range 30-89 years) with PVSG defined ET with platelet counts between  $615-2450 \times 10^9/L$  [30]. The vascular occlusive symptoms at presentation in 19 patients were painful feet and toes (erythromelalgia)

in 20, foot/leg ulcers in 4, atypical transient neurologic or ocular ischemic manifestations in 11, major thrombosis in 8. Hemorrhagic thrombocythemia (HT) had occurred in 8 at platelet counts in excess of  $1000 \times 10^9/L$ . Reduction of platelet count by low dose busulphan to less than  $400 \times 10^9/L$  and no aspirin resolved all vascular occlusive disease and the disappearance of bleeding [30]. Cox regression analysis revealed two prognostically important features in ET: age had a strong inverse correlation with survival and vascular occlusive symptoms correlated with a better survival. The number of death was 2.1 times than of a comparable control group and death from thrombosis was not significantly different from the number expected [30]. The natural history was featured by progression of ET into PV occurred in 9% and to myelofibrosis in 24% with death from myelofibrosis markedly increased [30].

From 1974 to 1986 the Rotterdam MPN Working Group treated 20 symptomatic ET patients with aspirin and one course of busulphan. The symptomatic microvascular thrombotic circulation disturbances ranged from erythromelalgia (N=18), atypical and typical transient ischemic attacks (N=6), and acute coronary syndromes (N=3) were [31,32]. Mean platelet count before busulphan treatment was  $1009 \times 10^9/L$ , (range 545 to  $>1525$ , Figure 13). One course of busulphan treatment was started at a dose of 4 to 6 mg/day at platelet counts  $>1000 \times 10^9/L$  (Figure 10). At platelet count just below  $1000 \times 10^9/L$  busulphan dose was reduced to 4 or 2 mg/day under careful blood cell

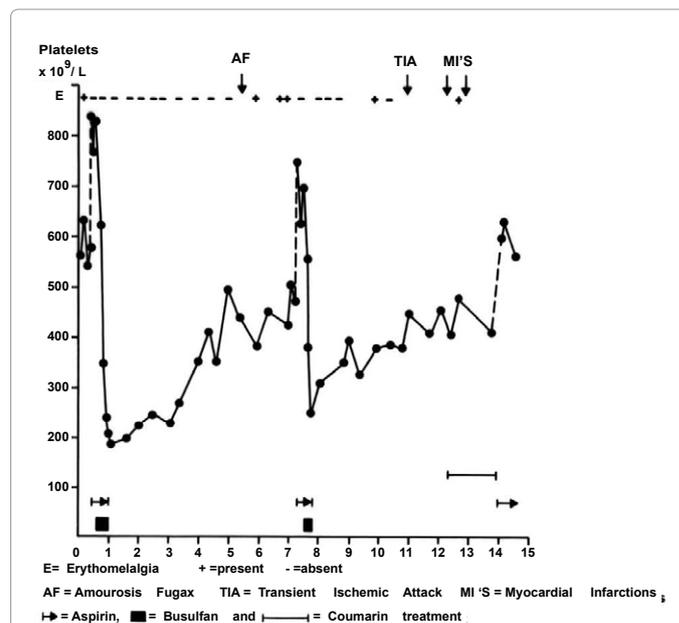
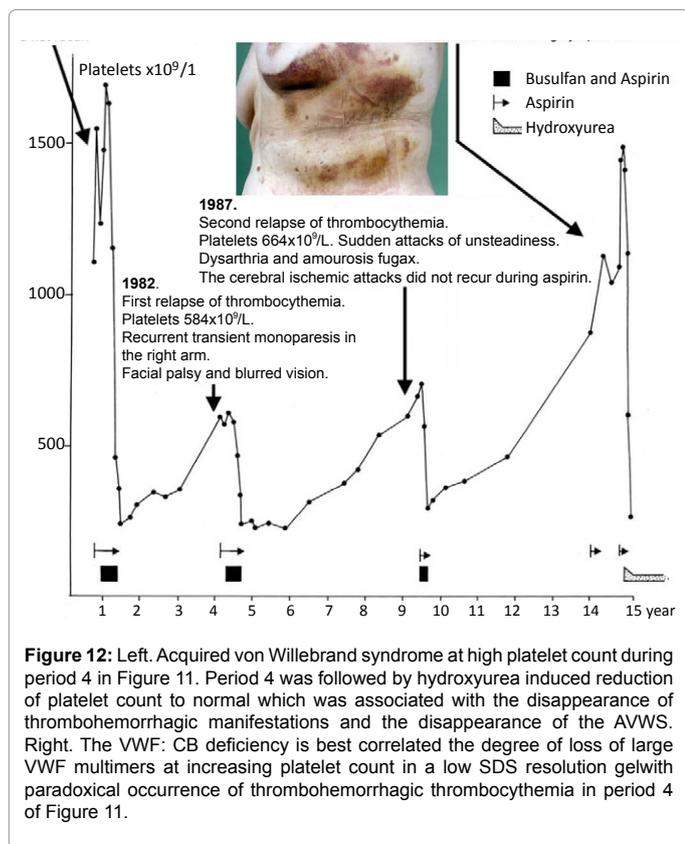


Figure 11: A 72 year-old woman with a three years history of burning painful red or blue toes and forefoot sole of the right foot (erythromelalgia) presented in December 1978 with spontaneous hemorrhages of large ecchymoses on the chest and subcutaneous hematotomas. Paradoxical occurrences of platelet-mediated thrombosis and bleeding in this case with thrombocythemia (ET with features of PV in the bone marrow: prodromal PV) 1978 peak 1 in the figure: first episode of erythromelalgia for 3 years followed by simultaneous occurrence of thrombotic thrombocythemia and hemorrhagic thrombocythemia at platelet counts around  $1500 \times 10^9/L$  in 1982 and 1987. During periods of busulphan induced remission of thrombothemia (platelet counts below  $400 \times 10^9/L$ ) the patients was asymptomatic when not on aspirin. Peak 2 and 3 in the figure: recurrence of second and third episode of thrombotic thrombocythemia at platelet counts between 600 and  $800 \times 10^9/L$ . 1992 peak 4 with a fourth episode of thrombotic and hemorrhagic thrombocythemia at platelet counts of  $1040 \times 10^9/L$  and aspirin-induced increase to around  $1500 \times 10^9/L$  associated with acquired von Willebrand factor deficiency type 2A (acquired von Willebrand syndrome: AVWS (figure 12).



counts every week. Busulphan was discontinued in all 20 ET cases when platelet counts reached the upper limit of normal ( $350 \times 10^9/L$ ) (Figure 13). At time of complete remission of ET, aspirin was discontinued to test the hypothesis whether control number to normal was associated with cure and no recurrence of erythromelalgic circulation disturbances. As shown in Figure 9 the mean platelet count before and after busulphan treatment was  $1009 \times 10^9/L$  (range 545-1525) and 241 (range 159-315)  $\times 10^9/L$  respectively. At time of normal platelet count ( $<350 \times 10^9/L$ ) erythromelalgia did not recur after discontinuation of aspirin. The total dose burden of only one course of busulphan treatment ranged from 106-484 (mean 315) mg. The duration of the busulphan course ranged from 44 to 218 (mean 116) days. All 20 patients with busulphan induced remission of ET remained asymptomatic after discontinuation of both busulphan and aspirin as long as platelet counts were normal for a follow-up period from 10 to 60 months (Figure 10). At time of relapse of ET (platelets  $>400 \times 10^9/L$ ) in 12; 8 became symptomatic (mainly erythromelalgia and atypical TIA in a few) at slight increased platelet count of 410, 450, 490, 500, 515, 545, 548 and  $635 \times 10^9/L$  indicating the need of low dose aspirin (100 mg), and 4 remained asymptomatic at platelet counts of 577, 600, 648 and  $725 \times 10^9/L$  (Figure 10). Based on these prospective intervention studies, we decided in 1986 to treat our ET patients with aspirin at time of presentation, during follow-up, and at relapse as long as platelet counts were between 400 and  $1000+200 \times 10^9/L$ . In 1986, hydroxyurea at that time became the drug of choice for clear indications to reduce platelet count from above to below  $1000 \times 10^9/L$  with continuation of low dose aspirin (Tables 4 and 5). At that time bleeding symptoms, aspirin side effects and platelet counts in excess of 1000 to  $1500 \times 10^9/L$  were clear indications for correction of platelet counts in ET and PV patients [10,31,32].

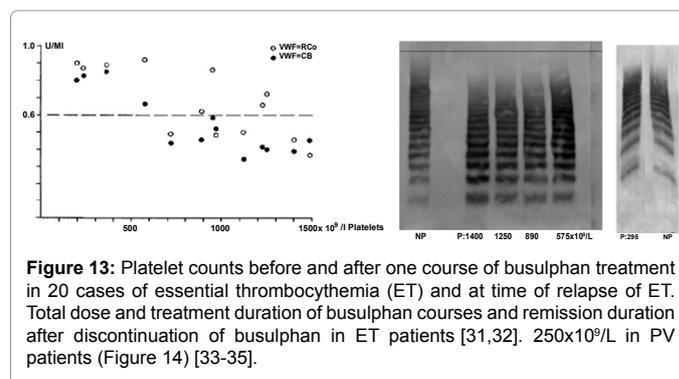
## The Spectrum of ETT and HT in ET and PV patients: Therapeutic Implications

In 1980 [33-35] Michiels reviewed 100 case histories of primary hemorrhagic thrombocythemia (HT) and 99 cases of erythromelalgic thrombotic thrombocythemia (ETT, 67 ET and 32 PV). The bleeding manifestations in 100 HT cases ranged from gastrointestinal chronic occult blood loss, melena and hematemesis to mucocutaneous bruises, hematomas, ecchymoses, gum bleedings and secondary bleeding [33-35]. HT was usually associated with significant leukocytosis and splenomegaly and the platelet count at time of bleeding in 100 HT cases ranged from 800 to above  $4000 \times 10^9/L$ . The manifestations in 99 ETT (67 ET and 32 PV) patients included erythromelalgia, acrocyanosis, digital gangrene, amaurosis fugax, transient ischemic attacks, stroke, angina pectoris and myocardial infarction, superficial thrombophlebitis and deep vein thrombosis. The platelet count at time of ETT in ET and PV patients ranged from 400 to  $2000 \times 10^9/L$  in ET patients and from 350 to 1

ETT=Erythromelalgic Thrombotic Thrombocythemia.  
HT=Hemorrhagic Thrombocythemia; ET: Essential Thrombocythemia;  
PV=Polycythemia Vera.

VWF:RCO=Von Willebrand Ristocetine Cofactor Activity; VWF:CB=Von Willebrand Factor Collagen Binding.

The final follow-up of the Rotterdam MPD Working Group (1975-1995) on the efficacy and safety for the prevention and treatment of thrombotic complications in 68 ET patients seen in the Academic Hospital Rotterdam has been critically evaluated by Perry van Genderen et al (1997) [36,37] independent from the principal investigator (Michiels). In this prospective observational Rotterdam



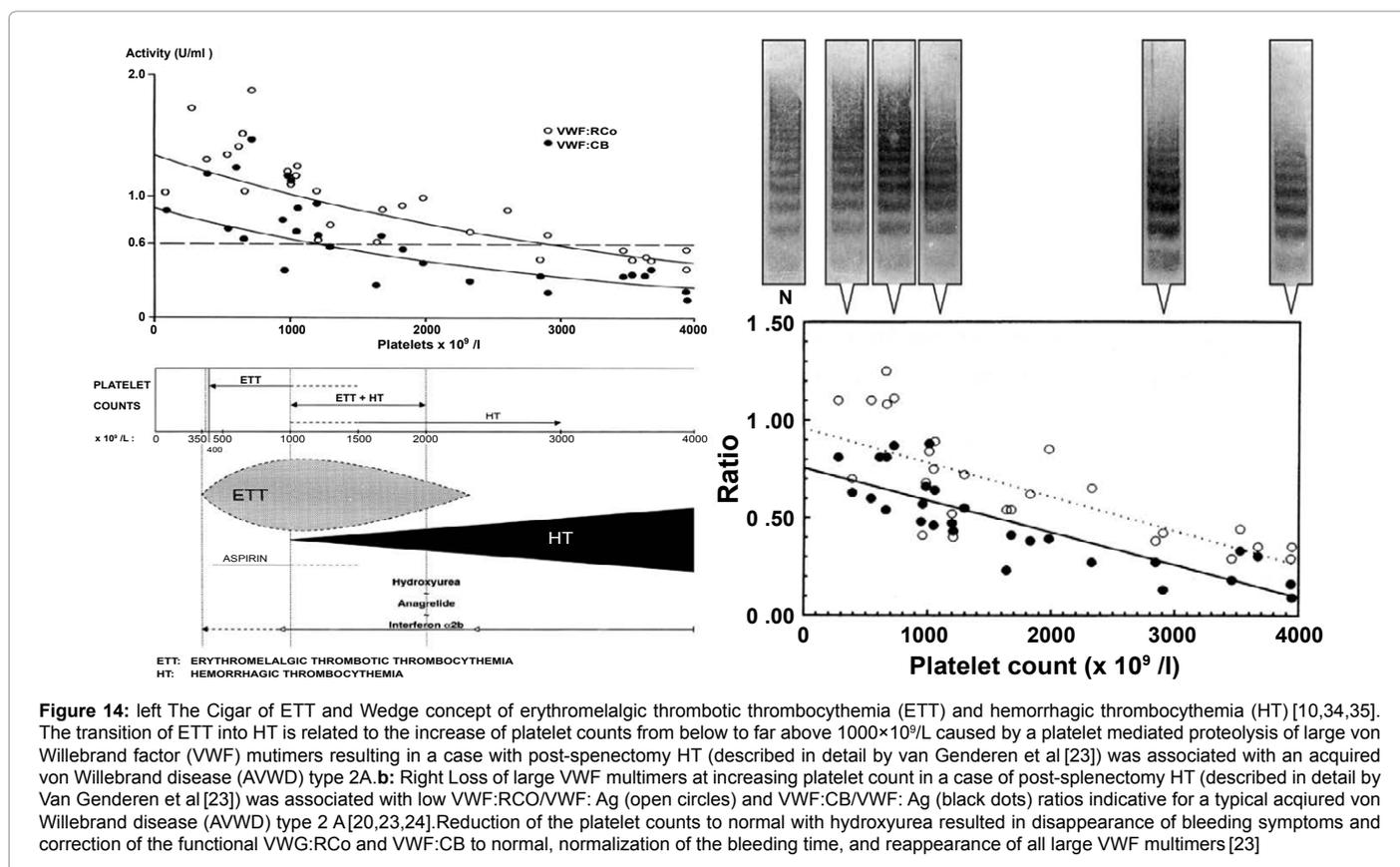
Treatment Strategy	Thrombotic complications		Bleeding complications		
	Person/ yrs	Events (n)	person-years	Events (n)	person-years
Asymptomatic 14 Patients:					
Watchful waiting	127	27*	33.3	2	1.6
Symptomatic 54 patients:					
Low-dose aspirin	139	5	3.6	10***	7.2
Platelet reduction	113	10**	8.9	2	1.8
Low-dose aspirin +	40	0	-	4	10
platelet reduction					
Total		419	42	18	

**Table 4:** Incidence of thrombotic and bleeding complications in the prospective 1975-1996 Rotterdam study of 68 ET patients during a median follow-up of 6.7 years according to treatment strategy (Van Genderen et al 1997) [36,37]. Mean platelet count  $\bar{=}$ 610, range 410-831  $\times 10^9/L$  at time of thrombotic event.  $\bar{=}$ 624  $\pm$  255  $\times 10^9/L$  at time of thrombotic event.  $\bar{=}$ 1737, range 661-3460  $\times 10^9/L$  at time of bleeding event (Table 5).

Platelets	Platelets	Platelets	Platelets
400-1500x10 <sup>9</sup> /L	400-1000x10 <sup>9</sup> /L	400-1000x10 <sup>9</sup> /L	>1500x10 <sup>9</sup> /L
Low risk ETT/HT	Standard risk ETT	High risk ETT/HT	High ETT/HT risk
<b>JAK2 wild type</b>	<b>JAK2<sup>V617F</sup> mutated</b>	<b>JAK2<sup>V617F</sup> mutated</b>	<b>All variants ET</b>
Asymptomatic Microvascular disturbances	Microvascular disturbances only	Major thrombosis Spontaneous bleeding or elicited by aspirin	>1000 x10 <sup>9</sup> /L and minor thrombosis/bleeding=high
No vascular risk	No vascular risk	Vascular risk(s)	No vascular risk
No bleeding risk	No bleeding risk		Bleeding risk
All ages	All ages	Age >65 years	All ages
Aspirin uncertain Wait and see?	Low dose aspirin 50 to 100 mg/day	Platelet reduction to normal or near normal	Platelet reduction to <1000x10 <sup>9</sup> /l
Intermediate risk			
Aspirin primary prevention? <b>ET patients and their physician usually prefer the use of low dose aspirin</b>	Microvascular disturbances and platelet count between 1,000 and 1,500 x10 <sup>9</sup> /l with clear indication aspirin*, →side effects <b>platelet reduction</b>	Continue aspirin*	<b>When platelets &lt;1,000 x 10<sup>9</sup>/l continue or add aspirin</b>

\*At platelet counts in excess of 1,000+250x10<sup>9</sup>/l aspirin will usually elicit bleeding symptoms, which disappear after reduction of platelet counts to below 1,000 x 10<sup>9</sup>/l with continuation of aspirin (Figure 4). If side effects of aspirin (gastritis, GI-bleeding occur) reduce platelet to normal and consider indomethacin 25 mg TID.

**Table 5:** Low, intermediate and high thrombohemorrhagic risk stratification of ET, ET with features of early PV in blood and bone marrow (prodromal PV) and classical PV patients: a flexible approach towards therapeutic implications with reference to platelet counts [11]. At platelet counts in excess of 1,000+250x10<sup>9</sup>/l aspirin will usually elicit bleeding symptoms, which disappear after reduction of platelet counts to below 1,000 x 10<sup>9</sup>/l with continuation of aspirin (Figure 4). If side effects of aspirin (gastritis, GI-bleeding occur) reduce platelet to normal and consider indomethacin 25 mg TID.



ET study the 68 consecutive ET patients were followed between 1975 to 1995 for a total of 419 patient years (Table 4) [36,37]. At time of presentation 54 patients had ET related microvascular thrombotic complications at platelet counts between 575 and 1031, mean 750x10<sup>9</sup>/L and treated with low-dose aspirin, a platelet lowering agent (busulfan or hydroxyurea) or both. The 14 ET asymptomatic ET patients suffered from 27 thrombotic and two bleeding episodes occurred during a follow-up of 127 patient years; platelet counts at time of thrombotic

event were between 410 and 831, mean 610x10<sup>9</sup>/L. Only four of the initially 14 asymptomatic ET patients remained asymptomatic after at least 30 person-years follow-up. In the treatment group of low-dose aspirin alone, 5 thrombotic events during a follow-up of 179 patient years, and 10 bleeding episodes occurred in ET patients receiving low dose aspirin monotherapy during a follow-up of 139 patients years at platelet counts between 661 and 3460 (mean 1737)x10<sup>9</sup>/l (Table 4). The main conclusion from this observation is that aspirin alone in ET at

platelet counts above  $1000 \times 10^9/l$  in general daily practices is not safe enough in terms of bleedings elicited by aspirin indicating the need to add one of the non-leukemogenic platelet lowering agents pegylated interferon or anagrelide [36,37]. In patients receiving platelet lowering agents either busulfan or hydroxyurea and no aspirin, 10 thrombotic complications occurred during follow-up of 40 patient years at platelet counts above  $400 \times 10^9/L$  ( $624 \pm 25 \times 10^9/L$ , Table 4), indicating that inadequate control of platelet number in thrombocythemia of ET and PV patients is associated with a persistent high incidence of microvascular disturbances including MIAs when not on aspirin at platelet counts above 350 to  $400 \times 10^9/L$  (Figure 14). In this landmark study of Van Genderen et al, age above 65 years was not a risk factor for thrombotic recurrences in ET when on low dose aspirin [36,37]. In the 1986 London study of 37 ET cases treated with low dose busulfan for the treatment of ETT and HT not on aspirin (mean age 60.5, range 30-89 years, platelet between  $615-2450 \times 10^9/L$ , Figure 14) [25] all vascular occlusive disease and bleeding resolved at time of platelet correction to normal [30]. Age had a strong inverse correlation with survival but vascular occlusive symptoms correlated with a better survival; progression of ET into PV occurred in 9% and to myelofibrosis in 24% with death from myelofibrosis markedly increased [30].

The therapeutic implications from the prospective Rotterdam ET studies are completely in line with the concept that microcirculatory thrombotic complications in thrombocythemia already occur at platelet counts in excess of  $400 \times 10^9/L$ , which are relieved by reduction of platelet counts to normal ( $<400 \times 10^9/L$ ) or by control of platelet function with low dose aspirin (Figures 14 and 15). Thrombocythemia with platelet counts in excess of  $1000+250 \times 10^9/L$  are frequently associated with the paradoxical occurrence of thrombosis and bleeding. Mucocutaneous bleedings spontaneously occur at platelet count in excess of  $1000+250 \times 10^9/L$  due to an acquired type II-like von Willebrand syndrome (AVWS, absence of high and intermediate von Willebrand factor (VWF) multimers) increasing in severity at increasing platelet counts to high levels above 1000 to  $1500 \times 10^9/L$  (Figures 14 and 15). Reduction of platelet count by anagrelide, interferon (or hydroxyurea if anagrelide and interferon fail) to less than  $1000 \times 10^9/L$  will result in the persistence of microvascular thrombotic events and the disappearance of the bleeding symptoms and AVWS (Figures 14 and 15). Correction of the platelet counts to normal is associated with no recurrences of microvascularevents and complete correction of the VWF-multimeric pattern and correction of all VWF-parameters to complete normal values. The study of Michiels et al. [3-11,31-34] and Van Genderen et al. [23-27,35-57] served as the scientific rationale for the ECLAP (European Collaboration on Low-dose Aspirin in PV) study design (1997) [33]. The ECLAP study nicely confirmed our concept of the effectiveness and safety of low dose aspirin in ET and PV in particular [38,39]. One low dose aspirin reduces the risk of microvascular ischemic or thrombotic complications from above 50% to less than 5% during 6.2 years of follow-up, and thereby prevented the progression from low thrombotic risk to high risk thrombotic in ET and PV [38,39].

### Low, Intermediate and High Thrombotic Risk in ET and PV Not on Aspirin

The risk stratification for thrombosis as low, intermediate and high thrombotic risk by Cortelazzo et al. in 1990/40 has been derived from a historical cohort of 100 ET patients not treated with aspirin thereby overlooking the original and updated key references of Michiels et al. [3,4,11,31-38,41] on the demonstration of aspirin responsive platelet-mediated arteriolar and arterial thrombotic tendency inherent to thrombocythemia in ET and PV patients.

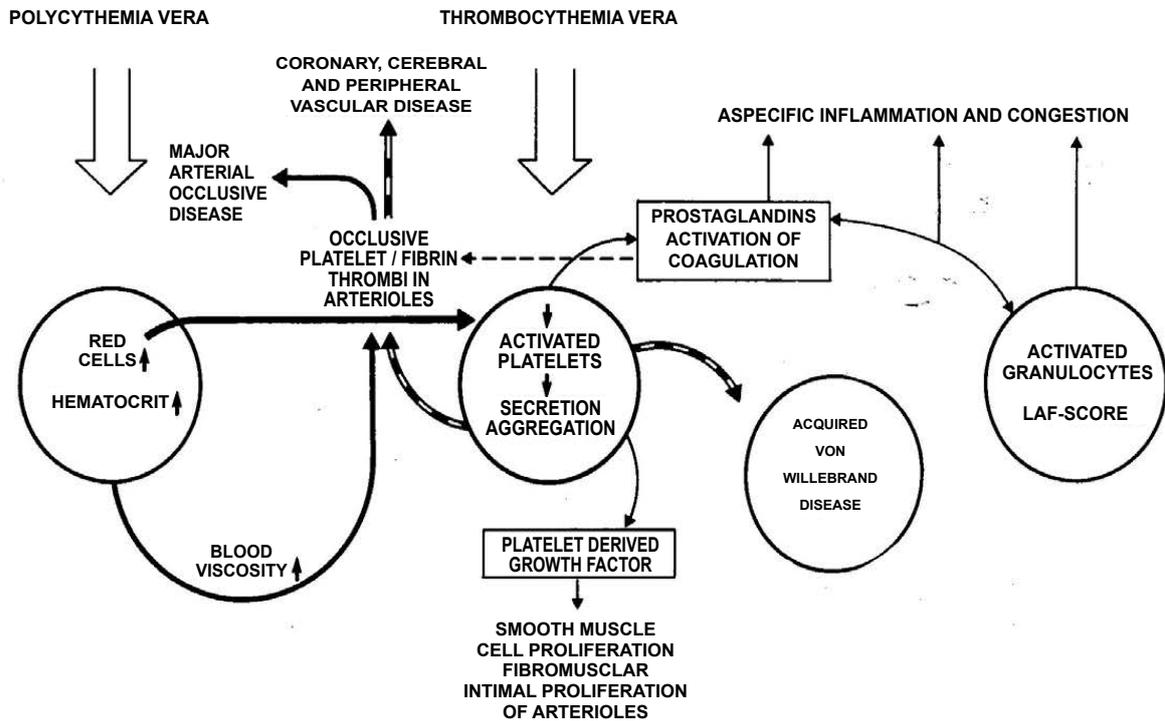
The characteristics of the thrombotic events in the retrospective Bergamo cohort of 100 patients were the following:

The age distribution of this cohort of ET did not reflect real life experience since the number of young ET patients was artificially manipulated to one third in the young age group to reach statistical significance. The risk for thrombotic complication was artificially low (1.7%) in thrombocythemia at young age below 40 years, but was high at age of >60 years (15%) not on aspirin, and moderately increased (6.3%) in the age group of 40 to 60 years, which is not significantly different from the thrombotic risk of 15% in the age group of >60 years [40]. Please note that in the Dutch prospective ET and PV studies the risk of thrombosis in untreated ET and PV is not age dependent and causally related to platelet-mediated thrombosis in the various stages of prefibrotic and early fibrotic MPN disease of ET and PV patients [36,37,40]. The type and number of 25 arterial and 3 venous thrombotic episodes in 20 out of a historical cohort of 100 untreated ET patients in the 1990 Bergamo study were mainly microcirculatory events including digital ischemia, transient ischemic attacks, superficial thrombophlebitis, unusual site of DVT, no stroke, and major thrombosis only in 4, myocardial infarction in 3 and femoral DVT in 1 (Table 6) [40]. It has been demonstrated at that time that such microcirculatory disturbances, superficial thrombophlebitis and TIAs are highly sensitive to low dose aspirin but not to coumarin [2]. If left untreated symptomatic ET patients with microcirculatory disturbances including erythromelalgia and atypical TIAs or visual symptoms are supposed to be at very high risk for digital ischemia, TIAs, stroke or acute coronary ischemic syndromes [1-7]. We concluded in 1995 [36] and 1997 [37] that the stratification in low, intermediate and high thrombotic risk in the 1990 retrospective Bergamo study [40] has been derived from ET patients not on aspirin. The microvascular and major thrombotic complications in MPN-T disease of ET and PV patients at time of presentation and during follow-up while not on aspirin is independent of the degree of thrombocytosis or MPN disease burden in terms of splenomegaly, leukocytosis and constitutional symptoms. This means that the stratification in low, intermediate and high thrombotic risk in the retrospective Bergamo ET study is based on statistical misinterpretation and mystification leading to authoritative overtreatment recommendation with hydroxyurea for MPN disease

Age	number of patients	Patient/years	Events number	Events % pt/yrs
<40 year	34	118	2	1.70%
40-60 years	37	112	7	6.30%
>60 years	29	73	11	15%
Total events in 20 of 100 ET patients=20%				

Cortelazzo et al. 1990		Number of patients	Number of events
Total		20	32
Arterial		17	25
Micro:	digital ischemia		7
	transient ischemic attacks		15
Major:	stroke		0
	myocardial infarction		3
Venous	superficial	3	7
	thrombophlebitis		3
	femoral DVT		1
	Unusual localization DVT		3
Bleeding complications		4	

**Table 6:** The type and number of microvascular thrombotic events in the 1990 Bergamo study are very characteristic for untreated thrombocythemia [3].



PATHOPHYSIOLOGY OF ARTERIOLAR AND ARTERIAL THROMBOSIS AND INFLAMMATION AS MULTICELLULAR PROCESSES IN THROMBOCYTHEMIA AND POLYCYTHYMIA VERA

ISH BASEL 1991  
MICHELS / VAN VLIET

**Figure 15:** Concept of erythromelalgic thrombotic thrombocytopenia (ETT) and hemorrhagic thrombocytopenia (HT) in patients with essential thrombocytopenia (ET) and polycythemia vera (PV). The broad spectrum of erythromelalgic thrombotic thrombocytopenia in essential thrombocytopenia (ET) and polycythemia vera (PV), which reflects a novel distinct disease entity of aspirin-responsive arterial thrombophilia in thrombocytopenia of various myeloproliferative neoplasms (MPN) [41]. The platelet-mediated microvascular thrombophilia aggravates into major arterial thrombotic events in PV patients when not on aspirin during follow-up. Correction of the hematocrit to around or below 0.40 is associated with significant reduction of major thromboses in PV but with the persistence of the microvascular syndrome of associated thrombocytopenia, which can best be prevented and treated by low dose aspirin but not by warfarin. At rising platelet counts to above  $1000 \times 10^9/L$  thrombocytopenia of various MPN is complicated by spontaneous mucocutaneous hemorrhages caused by acquired von Willebrand disease type 2A (Figure 14) [22,23].

in ET and PV patients 42. The so-called high thrombotic risk ET as defined by a history or presentation of thrombosis at time of diagnosis or by reaching the age 60 years is not in line with the real incidence of thrombotic risk of regularly treated according to good clinical practice in the Rotterdam ET and PV studies at that time and beyond up to 2013 [11]. In our analysis and experiences the high thrombotic risk and its persistence in the 2012 International Prognostic Score for ET (IPSET) [42] with the indication to treat ET disease with hydroxyurea when reaching the age of 60 years alone irrespective of the degree of MPN disease burden is not applicable anymore as targeted 2014 guideline for ET and PV patients on aspirin with a low or intermediate MPN disease burden. Clinicians should realise that high thrombotic risk in the 2012 IPSET guidelines result in overtreatment of ET and PV patients who have low or intermediate MPN disease burden.

The 1995 Bergamo study is a prospective randomized clinical trial (RCT) of 114 ET patients comparing hydroxyurea vs placebo in so-called high thrombotic risk ET patient [43]. This RCT is unbalanced since only 69% of the placebo group and 70% of the HU-treated ET patients received antiplatelet drugs, aspirin (effective in thrombocytopenia [3,4] or ticlopidine (not effective in thrombocytopenia [3,4]. The results show that 2 of 56 high thrombotic risk ET patients on hydroxyurea had major thrombotic events (one stroke, one myocardial infarction) and that 14 of 58 high thrombotic risk ET patients in the placebo group had

mainly minor thrombotic complications including microcirculatory disturbances in 12, and major thrombosis in 2 similar as in the HU arm (MI 1, and deep vein thrombosis in 1) [43]. Moreover, 10 of the 14 symptomatic patients in the placebo arm manifested mainly microvascular disturbances and were not on treatment with aspirin [43]. A direct comparison of HU reaching normal platelet counts vs. low dose aspirin alone in ET patients at platelet counts below or around  $1000 \times 10^9/L$  is predicted to be equally effective for the prevention of microvascular circulation disturbances in ET (Figure 14 and 15). In the 1995 Bergamo study, the use of aspirin in the placebo arms as compared to the HU arm was not associated with increased bleeding events at platelet counts around  $1000 \times 10^9/L$  [35]. The recommendation from this unbalanced RTC (HU vs placebo) to use hydroxyurea in so-called high thrombotic risk ET as first line treatment for ET is not justified and potentially leukemogenic and has led to significant world-wide over-treatment of ET patient who usually have low to intermediate MPN disease burden and a normal life expectancy [43].

The combined low dose aspirin platelet reduction strategy to lower platelet count to about values around  $600 \times 10^{12}/L$  (near normal platelet concept) in symptomatic ET-patients by anagrelide or, interferon (hydroxyurea when anagrelide and interferon failed) is most effective in daily practices for the treatment of thrombocytopenia in ET and PV [11]. Prospective studies comparing this concept of near normal

Patients	PV N=323		ET N=639	
	hetero	homozygous	hetero	wild type
JAK2V617F mutation status	hetero	homozygous	hetero	wild type
Number of patients	219	104	368	257
At diagnosis				
Major arterial events	21%	15.4%	21.70%	10.50%
Venous events	6.40%	2.90%	7.90%	4.70%
During 10 years follow-up (not on aspirin)				
Major arterial events	10.10%	12.50%	6.30%	5.80%
Venous events	4.10%	7.70%	6.30%	2.70%
Total during life time follow-up				
Major arterial	31.10%	27.90%	28%	16.30%
Venous	10.50%	10.60%	14.20%	7.40%

**Table 7:** Major cardiovascular events at diagnosis or during long-term follow-up in 323 PV and 639 ET patients according to the JAK2V617F mutation status in the retrospective study of Vannucchi [48]. Only major thrombotic events were retrospectively recorded not including the erythromelalgic peripheral and migraine like cerebral ischemic events [48]. In 14 homozygous ET patients total major arterial and venous events occurred in 78.6% and 57.1% respectively.

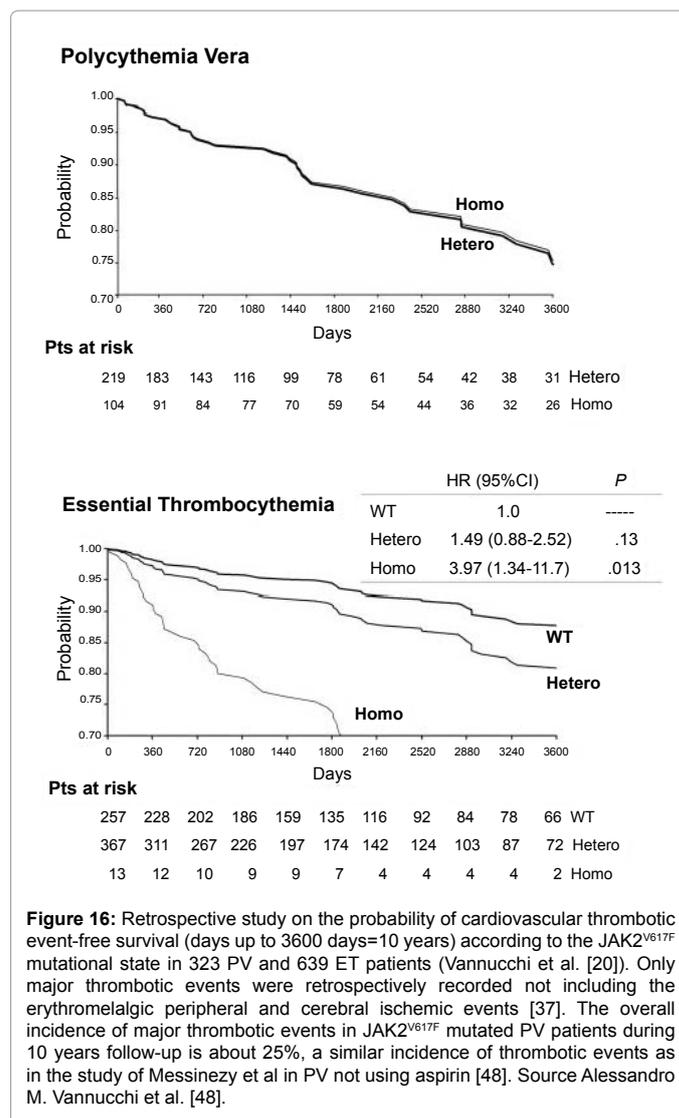
platelet count strategy versus low dose aspirin alone is warranted. The prediction is that the near normal platelet count strategy in symptomatic JAK2 mutated ET, and ET mimicking PV (prodromal PV) seems to be far superior to low dose aspirin alone, simple because low dose aspirin will significantly increase the risk of bleeding complications at platelet counts around and above 1000 x 10<sup>9</sup>/L (Figures 14 and 15).

### Thrombosis and Bleeding Risk in JAK2 Mutated ET and PV and JAK2 Wild Type ET

With the advent of molecular screening of MPN patients it should be realized that WHO-ET patients with a JAK2<sup>V617F</sup> mutation load of less than 50% of the granulocytes are indeed heterozygous for the JAK2 mutation [44-46]. However, WHO-PV patients with a JAK2<sup>V617F</sup> mutation load of less than 50% of the granulocytes proved to be combined heterozygous homozygous positive for the JAK2<sup>V617F</sup> mutation [44-46]. Vannucchi et al determined the JAK2<sup>V617F</sup> mutation allele burden as expressed in percentages of JAK2<sup>V617F</sup> mutated granulocytes of 173 PV patients at diagnosis [47]. The JAK2<sup>V617F</sup> allele burden in granulocytes ranged from 1-25% in 33%, from 25-50% in 29%, from 50 to 75% in 20% and from 75 to 100% in 18% and treatment consisted of phlebotomy in 49% and cytoreductive therapy (mainly hydroxyurea) in 51% [47]. The JAK2<sup>V617F</sup> allele mutation burden correlated with MPN disease activity in terms of stimulated erythropoiesis by higher hematocrit and erythrocytes, lower MCV, serum EPO and ferritin, and stimulated myelopoiesis by higher leukocytes, serum LDH and LAP score [47]. Comparing by statistics alone the PV patients with low JAK2<sup>V617F</sup> (1 to 50%) versus high JAK2<sup>V617F</sup> (50-100%) allele burden, the relative risks (RR) for MPN disease burden increased from 1 to 4 for pruritus, from 1 to 4 for palpable splenomegaly and from 1 to 4 for spleen sizes above 15 cm length diameter on scan [47].

In a subsequent retrospective study Vannucchi et al assessed in 2007 the incidence of thrombosis related to the JAK2 allele burden in a large retrospective study of 962 MPN-T patients subdivided in 323 PV and 639 ET patients [48]. Aspirin responsive platelet thrombophilia or microvascular symptoms due to microvessel disorder including migraine-like headache, acral paresthesia, erythromelalgia, transient neurological and visual disturbances (Sticky Platelet Syndrome 49) were excluded by definition and not considered in this retrospective analysis [48]. Major thrombotic events were assessed as could be objectively documented in the medical records including ischemic stroke, transient ischemic attacks, myocardial infarction, angina pectoris, deep vein thrombosis abdominal vein thrombosis, and pulmonary embolism

occurring at diagnosis or follow-up when not on aspirin [48]. According to these exclusion and inclusion criteria, the clinical profile and the incidence of major thrombotic events in 188 JAK2<sup>V617F</sup> homozygous MPN patients (JAK2<sup>V617F</sup> mutation above 50% in 104 PV and 14 ET) and in 587 heterozygous (JAK2<sup>V617F</sup> mutation less than 50% in 219 PV and 257 ET) and 257 wild type ET patients was assessed and calculated in Table 7 and Figure 16. Homozygous JAK2<sup>V617F</sup> positive patients with an allele mutation load above 50% were older, had higher leukocyte counts, hematocrits and larger spleen volumes indicating advanced MPN disease [48]. One hundred seventy-six patients (18.3%) had a major thrombotic event at diagnosis with a similar frequency in PV (19.2%) and ET (17.8%). A similar incidence was found in our analysis of the literature in 1241 ET patients not on aspirin from 14 retrospective studies [50]. During long-term follow-up, major thrombosis (usually not on aspirin) occurred in 122 patients (12.7%), corresponding to 14.9% in PV and 11.6% in ET patients and hemorrhages at diagnosis manifested in 55 (5.7%) patients, 5.3% in PV and 6.0% in ET [20]. The overall incidence of major thrombotic events during 10 years follow-up usually not on aspirin was about 20% in ET heterozygous for the JAK2<sup>V617F</sup> mutation and in about 10% for JAK2 wild type ET [48]. A hemorrhage during follow-up was recorded in 45 (4.7%) ET/PV patients. A similar incidence of hemorrhages was found in our analysis



PV: WHO-ECMP stage	0	1	2	3	4	5	6
WHO-ECMP	Prodromal masked PV	Erythrocy-themic PV	Early PV	Overt PV	PV PMF	Post-PV MF	Leukemic PV
Clinical Diagnosis	Prodromal masked PV	Erythrocy-themic PV	Early PV	Classical PV	Masked PV	Spent PV	MDS/AL
LAP-score	↑	↑	↑	↑	↑/↑↑	variable	variable
Red cell mass (RCM)	N	↑	↑	↑	↑	variable	N/↓
Serum EPO	N/↓	N/↓	↓	↓	↓	variable	N/↓
Erythrocytes x10 <sup>12</sup> /l	>5.8	<5.8	>5.8	>5.8	>5.8	variable	N/↓
Leukocytes x10 <sup>9</sup> /l	<12	<12	<15	< or >15	>15	>20	>20
Platelets x10 <sup>9</sup> /l	>400	,400	< or >400	>400	< or >1000	variable	variable
WHO-ECMP bone marrow	Early PV	Early PV	Early PV	Trilinear PV	Trilinear PV	Myelofibrosis	Leukemic
Bone marrow cellularity (%)	50-80	50-80	60-100	80-100	80-100	Decreased	Increased
Grading reticulin fibrosis: RF	RF 0-1	RF 0-1	RF 0-1	RF 0/1,	RCF 2/3	RCF 3/ 4	
Grading myelofibrosis: MF <sup>57</sup>	MF 0	MF 0	MF 0	MF 0	MF 1/2	MF 2/3	
Splenomegaly on palpation	No/+	No	No/+	+	++/+++	/large	large
Spleen size, echogram cm	<12-15	<13	15-Dec	18-Dec	18->20	>20	>20
Spleen size on palpation cm	0-3	NP	0-3	6-Apr	>6	>8	>8
JAK2 <sup>V617F</sup> in Granulocytes %	low	low	Moderate <50	High >50	High >50	High >50	
JAK2 <sup>V617F</sup> in BFU-e (exon 12)	+(++)	+(++)	+(++)	++	++	++	No or ++
Therapeutic implications	Low risk	Low risk	Low risk	Intermediate risk PV	High risk	Post-PV MF	Leukemia
Anno 2014	MPN	MPN	MPN	PV	PV-MF	Spent phase PV	
First line Aspirin/Phlebotomy	Aspirin	Aspirin	Phlebotomy	Phlebotomy*	If IFN resistant ◊	JAK2	Chemotherapy
Second line IFN versus	Phlebotomy	Phlebotomy	Aspirin	Aspirin	HU or JAK2	Inhibitor ◊	Bone marrow transplantation?
Hydroxyurea (HU)			Low dose IFN responsive	IFN resistant ◊ HU	inhibitor	Bone marrow	Supportive
Third line JAK2 inhibitor						transplantation	

**Table 8:** Staging of JAK2<sup>V617F</sup> positive prodromal PV, erythrocythemic PV, and five stages of PV according to WHO-ECMP criteria related to therapy anno 2013 [41] \*↑ = increased, ↓ = decreased, N = normal, + = present or heterozygous; ++ = homozygous.

of the literature in 1241 ET patients from 14 retrospective studies [50]. The frequency of bleeding was higher in JAK2<sup>V617F</sup> homozygous (21.4%) than in wild type or heterozygous ET patients, 3.1% and 3.8% respectively. The higher bleeding tendency in homozygous JAK2<sup>V617F</sup> MPN patients is predicted to be related to higher erythrocyte counts at increased platelet and leukocyte counts and its pathophysiology of the underlying mechanisms is currently under our investigation.

In the second Vannucchi study, a total of 214 patients were treated with phlebotomy, 58% of 219 PV and 4% of 257 ET patients. Myelosuppressive chemotherapy was administered to 497 patients (52%) including 59% of 219 PV and 48% of 257 ET patients [48]. For comparison, in the Dutch 2008 survey of 363 MPN (123 ET, 190 PV and 50 MF) patients 93% of PV, 71% of ET and 37% of MF were on aspirin mainly because of microvascular symptoms including migraine-like headache, acral paresthesia, erythromelalgia, transient neurological and visual disturbances. Phlebotomy became the first line treatment in 6% of ET, 78% of PV and 9% of MF when the Dutch recommendations are applied [51,52].

Because of advanced or symptomatic MPN disease 31% of ET, 29% of PV and 30% of MF were on treatment with hydroxyurea and 16% of ET, 16% of PV and 4% of MF were on treatment with pegylated interferon (PegasysR) [11,54,55]. The 20% difference of HU use (50% of Italian MPN-T patients versus 30% of Dutch MPN patients) can readily be ascribed to significant differences in the Italian IPSET recommendations 42 versus the Dutch guidelines for MPN disease in ET and PV patients [50-54].

## Hydroxyurea is Not an Innocent Myelosuppressive Agent in PV and ET

Hydroxyurea is not an innocent drug and should be used with

caution and withheld as long as phlebotomy and low dose aspirin are effective in the treatment of early and intermediate plethoric PV stages 1 and 2. In the 1980 French PV study in PV patients under the age of 65 years, toxicity of hydroxyurea was observed within 5 years in 29% of 133 PV patients, which was limited to dry skin and acne in 7%, gastric pairdiarrhea in 9%, aphthous ulcers in the mouth in 10%, and leg ulcers in 9% [56]. Leg ulcers only healed after discontinuation of hydroxyurea; these complications generally appear late within 5 years or more after initial treatment. Dry skin in 1, aphthous stomatitis in 4 and leg ulcers in 10 cases were the reasons for replacing hydroxyurea by pipobroman in 9%. In the update 10 years later in 1997, the incidence of leukemia was about 10% at 13 years in hydroxyurea treated PV-patients and life expectancy was 70% at 14 years as compared to 83.7% in age-matched controls [56]. The frequency of MF or spent phase PV in the update in 1997 was 17% at 10 years and 40% at 16 years. The final analysis of this 1980 French PVSG study of HU as the upfront first-line therapy in 136 evaluable PV patients younger than 65 years is published in 2011 [57]. The cumulative incidence (probability) of myelofibrosis (MF) at 10, 15 and 20 years was 15%, 24% and 32% in the HU arm and the cumulative incidence of AML/MDS at 10, 15 and 20 years was 7.3, 10.7% and 16.6% for HU treated PV patients. As shown in Table 8, high risk PV with advanced MPN-T disease in ET and PV patients in terms of high JAK2<sup>V617F</sup> allele burden, progressive MPN disease, splenomegaly and constitutional symptoms are candidates for myelosuppressive (hydroxyurea) or myeloreductive (JAK2 inhibitors) treatment [11,41].

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