

World Journal of *Hematology*

World J Hematol 2017 August 6; 6(3): 32-61



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World Journal of Hematology

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Quarterly Volume 6 Number 3 August 6, 2017

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World Journal of Hematology is now indexed in China National Knowledge Infrastructure (CNKI).

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NAME OF JOURNAL
World Journal of Hematology

ISSN
 ISSN 2218-6204 (online)

LAUNCH DATE
 June 6, 2012

FREQUENCY
 Quarterly

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 Fang-Fang Ji, Director
World Journal of Hematology
 Baishideng Publishing Group Inc
 7901 Stoneridge Drive, Suite 501,
 Pleasanton, CA 94588, USA
 Telephone: +1-925-2238242
 Fax: +1-925-2238243
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PUBLICATION DATE
 August 6, 2017

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Aspirin cures erythromelalgia and cerebrovascular disturbances in JAK2-thrombocythemia through platelet-cyclooxygenase inhibition

Jan Jacques Michiels

Jan Jacques Michiels, Department of Hematology, University Hospital Antwerp, Antwerp University, B-2650 Edegem, Belgium

Jan Jacques Michiels, Goodheart Institute Freedom of Science and Education in Nature Medicine and Health, 3069 AT Rotterdam, The Netherlands

Author contributions: Michiels JJ solely contributed to this paper.

Conflict-of-interest statement: None.

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Manuscript source: Invited manuscript

Correspondence to: Dr. Jan Jacques Michiels, MD, PhD, Professor, Goodheart Institute Freedom of Science and Education in Nature Medicine and Health, Erasmus Tower, Veenmos 13, 3069 AT Rotterdam, The Netherlands. goodheartcenter@upcmail.nl
Telephone: +31-62-6970534

Received: March 20, 2017

Peer-review started: March 24, 2017

First decision: April 18, 2017

Revised: July 3, 2017

Accepted: July 17, 2017

Article in press: July 17, 2017

Published online: August 6, 2017

essential thrombocythemia (ET) and polycythemia vera (PV) with thrombocythemia spontaneously activate at high shear in arterioles, secrete their inflammatory prostaglandin endoperoxides and induce platelet-mediated arteriolar fibromuscular intimal proliferation. Constitutively activated JAK2 mutated hypersensitive (sticky) platelets spontaneously aggregate at high shear in the endarteriolar circulation as the cause of aspirin responsive erythromelalgia and platelet arterial thrombophilia in JAK2-mutated thrombocythemia patients. Increased production of prostaglandin endoperoxides E2 and thromboxane A2 released by activated sticky platelets in arterioles account for redness warmth and swelling of erythromelalgia and platelet derived growth factor can readily explain the arteriolar fibromuscular intimal proliferation. Von Willebrand factor (VWF) platelet rich occlusive thrombi in arterioles are the underlying pathobiology of erythromelalgic acrocyanosis, migraine-like transient cerebral attacks (MIAs), acute coronary syndromes and abdominal microvascular ischemic events. Irreversible platelet cyclo-oxygenase inhibition by aspirin cures the erythromelalgia, MIAs and microvascular events, corrects shortened platelet survival to normal, and returns increased plasma levels of beta-TG, platelet factor 4, thrombomodulin and urinary thromboxane B2 excretion to normal in symptomatic JAK2-thrombocythemia patients. *In vivo* activation of sticky platelets and VWF-platelet aggregates account for endothelial cell activation to secrete thrombomodulin and sVCAM followed by occlusion of arterioles by VWF-rich platelet thrombi in patients with erythromelalgic thrombotic thrombocythemia (ETT) in ET and PV patients. ETT is complicated by spontaneous hemorrhagic thrombocythemia (HT) or paradoxical ETT/HT due to acquired von Willebrand disease type 2A at platelet counts above $1000 \times 10^9/L$ and disappears by cyto-reduction of platelets to normal ($< 400 \times 10^9/L$).

Abstract

Hypersensitive (sticky) platelets in JAK2-mutated

Key words: Aspirin; Wonder drug; Erythromelalgia; Cerebral vascular disturbances; Platelet cyclooxygenase;

Migraine

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Core tip: About seventy years after the synthesis by Hoffmann, acetyl salicylic acid (aspirin) has been discovered in the late 1970s as a wonder drug that cures erythromelalgia and migraine-like cerebral microvascular disturbances by irreversible blockage of platelet cyclooxygenase mediated arteriolar inflammation and thrombosis in JAK2-mutated thrombocythemia of patients with essential thrombocythemia (ET) and polycythemia vera (PV). The ADP (P2Y₁₂) receptor inhibitors ticlopidin and clopidogrel, other platelet inhibitors that do not affect platelet cyclooxygenase, and coumarin are ineffective in the treatment of erythromelalgia and cerebral vascular thrombotic complications in ET and PV.

Michiels JJ. Aspirin cures erythromelalgia and cerebrovascular disturbances in JAK2-thrombocythemia through platelet-cyclooxygenase inhibition. *World J Hematol* 2017; 6(3): 32-54 Available from: URL: <http://www.wjgnet.com/2218-6204/full/v6/i3/32.htm> DOI: <http://dx.doi.org/10.5315/wjh.v6.i3.32>

PLATELET AND ENDOTHELIAL CELL PROSTAGLANDIN METABOLISM IN THE 1970s

Aspirin (acetyl salicylic acid) and aspirin like drugs inhibited prostaglandin biosynthesis (Vane 1971)^[1] leading to impaired prostaglandin E₂ and thromboxane A₂ (TxA₂) synthesis in platelets (Smith and Willies 1970)^[2,3]. Human platelets do form and release prostaglandin (PG) E₂ and PGF_{2α} from the precursor arachidonic acid (AA) in platelets released from the platelet membrane phospholipids in thrombin stimulated platelets^[2,3]. AA (0.5 mmol/L) causes aggregation of platelets in platelet-rich plasma (Silver *et al*^[3] 1973). AA in low amounts (0.1 mmol/L) enhance platelet aggregation induced by ADP, collagen or epinephrin was observed^[4]. AA (0.5 mmol/L), thrombin (1 μ/mL), collagen (80 μL), epinephrin (50 μmol/L) or ADP (50 μmol/L) induced equally amounts of radioactivity released from platelets preincubated with C¹⁴-serotonine or C¹⁴-adenine (Figure 1)^[4]. Large amounts of PGE₂ and PGF_{2α} are formed in platelets in response to AA, but small amounts of PGE₂ and PGF_{2α} are released from platelets in response to thrombin, collagen and epinephrin (Figure 1) indicating that shear stress induce spontaneous platelet activation produces large amount of platelet prostaglandin endoperoxides G₂, H₂, D₂ and E₂, which in retrospect proved to induce the inflammatory signs of erythromelalgia in thrombocythemia patients (Figures 2 and 3)^[4]. The

inhibiting effect of aspirin on platelet aggregation persisted a few days due to its irreversible inhibition of platelet cyclooxygenase activity^[4]. We used the method of Smith *et al*^[5] (1976) and measured the production of malodialdehyde (MDA) in platelet rich plasma after incubation with N-ethylmaleimide (NEM) as a measure for the degree of inhibition of cyclooxygenase activity and prostaglandin production in platelet (Figure 3). At that time in 1976 we discovered that aspirin cures erythromelalgia in thrombocythemia of ET and PV patients by irreversible inhibition of platelet cyclooxygenase activity as measured by the degree MDA inhibition in platelet rich plasma (Figure 3).

Hemler *et al*^[6] (1976) purified cyclooxygenase that forms prostaglandins. Moncada *et al*^[7] and Vane *et al*^[8] (1976) isolated the enzyme prostaglandin synthetase in endothelial cells from arteries that transformed cyclic endoperoxides to prostacyclin, that strongly inhibit platelet aggregation (Figure 2, 1976 concept of Michiels and Van Vliet). AA is metabolized in platelets and endothelial cells by cyclooxygenase to unstable cycloendoperoxides PG₂ and PGH₂, which in turn is broken down to the stable prostaglandins PGE₂, PGF_{2α} and PGD₂ (Figure 2)^[7,8]. The cyclic endoperoxides in EC are metabolized by prostacyclin synthetase into unstable PGI₂ (a strong platelet aggregation inhibitor) and its stable inactive endproduct. The cyclic endoperoxides in platelets are metabolized by thromboxane synthetase into the unstable thromboxane A₂ (a strong platelet aggregation agonist) and its stable inactive thromboxane B₂ (Figure 2). Several reports between 1975 and 1980 confirmed prostacycline formation in endothelial cells (ECs) of the vessel wall. The formation from platelet membrane phospholipids of arachidonic acid (AA) is the substrate for cyclooxygenase to synthesize prostaglandin endoperoxides in endothelial cells and platelets (Smith *et al*^[5] 1976, Moncada and Vane in 1979, Figure 2)^[9-19]. Thromboxane A₂ produced by platelets has vasoconstrictive and platelet aggregation stimulating properties, whereas prostacycline produced in endothelial cells (EC) causes vasoconstriction and strongly inhibits platelet aggregation (Figure 2). Half life times of prostacycline is two to three minutes. Half life time of thromboxane A₂ is a few hours and broken down to its endproduct thromboxane B₂, which is secreted by the kidney. Prostacycline is broken down into prostaglandin 6-keto-PGF-1-α (Figure 2). Prostacycline inhibit platelet aggregation through stimulation of adenyl cyclase and subsequent increase of cyclic AMP concentration in platelets (Figure 2). Thromboxane A₂ induce platelet aggregation through inhibition of adenyl cyclase and subsequent decrease of cyclic AMP in platelets (Figure 2). Prostacycline plays an important physiological role in the prevention of platelet adhesion and aggregation to the intact vessel wall^[13-15]. Disturbance of the balance between thromboxane A₂ from platelets and prostacycline from ECs plays an important role in the pathogenesis of arterial thrombosis by activation of

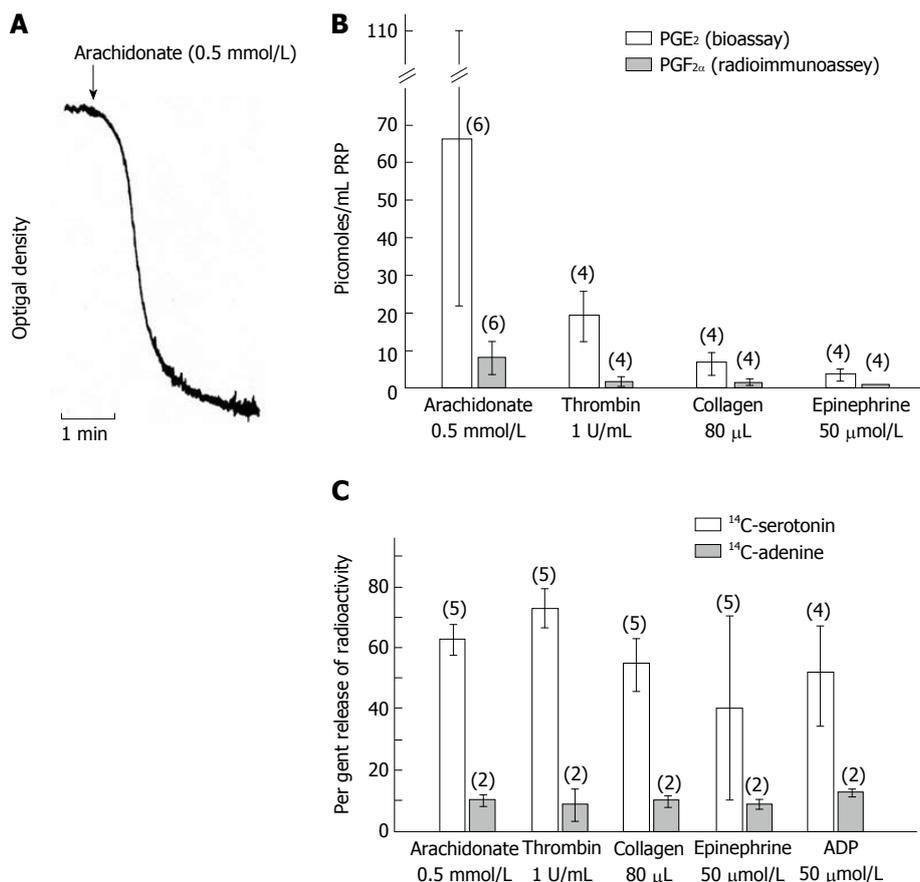


Figure 1 Arachidonic acid-induced human platelet aggregation and prostaglandin formation (Silver *et al*³¹ 1973). Arachidonate acid (AA), 0.5 mmol/L induces a normal platelet aggregation curve (A). AA induces secretion of large amounts of prostaglandins after platelet aggregation, but little or no prostaglandins secretion occur after platelet aggregation induced by thrombin, collagen and epinephrin (B). AA, thrombin, collagen, epinephrine and ADP induce aggregation and secretion of rather equal amounts radioactivity from PRP preincubated with ¹⁴C-serotonin or ¹⁴C-adenine (C). These findings implicate that spontaneous *in vivo* shear induced aggregation of sticky JAK2 mutated platelet in the endarteriolar circulation is associated with high prostaglandin levels as the cause of the inflammatory pain and signs (redness, warmth and congestion) of erythromelalgia in JAK2-mutated thrombocythemia (Michiels *et al*⁵³ in 1985 and Michiels⁸⁰ in 2017).

platelet aggregation on damaged endothelial cells of the arteriosclerotic vessel walls.

Arachidonic (AA) stimulated platelets produce large amounts of prostaglandin endoperoxides PGE₂, PGF_{2α}, and thromboxane A₂ (TxA₂) and small amounts of prostaglandin D₂ (Figure 1)^{1,13}. Prostaglandine E₂ is able to induce pain and inflammatory manifestations. Prostaglandin D₂ has platelet aggregation inhibitory activity through stimulation of adenylycyclase¹⁹. In the absence of thromboxane A₂ formation through irreversible inhibition of cyclo-oxygenase by aspirin, high concentrations of collagen, ADP and thrombin are still capable to induce platelet aggregation both *in vitro* and *in vivo* indicating that aspirin treated platelets retain their capability to adhere to subendothelium and aggregate in pathological situation like wound and arteriosclerotic vessel wall lesions. Secretion of the dense bodies contents ADP, ATP, calcium and serotonin during platelet activation subsequently propagate platelet aggregation, whereas serotonin also has vasoconstriction properties (Figure 1). During platelet aggregation alpha granules release platelet specific proteins like beta-thromboglobulin (beta-TG), platelet factor 4 (PF4), and platelet derived growth factor

(PDGF), of which the latter stimulates proliferation of the smooth muscle cells in the media of arterioles and vessels^{20,21}.

ASSOCIATION OF ERYTHROMELALGIA AND THROMBOCYTHEMIA IN PV AND ET

The association of erythromelalgia and PV was known for a long time²²⁻²⁶. Oppenheimer recognized that the erythromelalgia in PV frequently progressed into acrocyanotic digital ischemia or gangrene diagnosed as thromboangiitis obliterans²³. Dameshek and Henthel described a PV case with frequent episodes of erythromelalgia complicated by gangrene of the third toe suggestive diagnosed as thrombo-angiitis obliterans during longstanding follow-up of the PV: Hemoglobin 115%, erythrocytes $7 \times 10^{12}/L$ (normal value less than $6 \times 10^{12}/L$), white blood cells $19 \times 10^9/L$, and platelets $2850 \times 10^9/L$ ²⁷. Another PV case of Dameshek and Henthel²⁷ suffered from recurrent episodes of severe erythromelalgia since 5 years before PV could be diagnosed: Hemoglobin 116%,

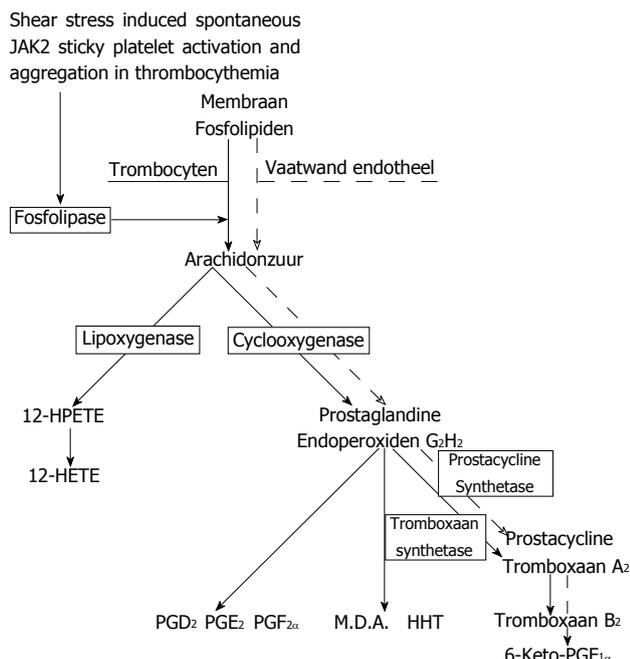


Figure 2 Dutch design by Michiels and Van Vliet on membrane phospholipid - arachidonic acid metabolism in platelets as compared to endothelial cells conceptualize in 1976, three years before the Moncada and Vane^[13] publication in the *NEJM*. Arachidonic acid (AA) is metabolized by lipoxygenase into (12-HPETE) and 12 HETE, and by platelet cyclooxygenase into prostaglandin endoperoxides G₂ and H₂, which consist of PGE₂, PG₂α, PGD₂, malondialdehyde (M.D.A.) and HHT. Prostaglandin endoperoxides in platelets are metabolized by thromboxane synthetase into thromboxane A₂. Thromboxane A₂ is potent inducer of platelet aggregation and smooth muscle cell contraction. AA induced prostaglandin endoperoxides in endothelial cells are metabolized by prostacyclin synthetase into prostacyclin. Endothelial cell (EC) derived prostacyclin causes vasodilatation and prevents platelet aggregation and platelet derived thromboxane causes vasoconstriction and platelet aggregation. Prostacyclin is continuously produced by endothelial cells, which have a nucleus to synthesize cyclo-oxygenase and prostacyclin. The biological half life times of prostacyclin and thromboxane A₂ are short and broken down to the inactive metabolites 6-keto-PGF₁-α and thromboxane B₂, which are secreted by the kidneys into the urine (Figure 15). Ticlopidine and clopidogrel inhibit ADP induced platelet aggregation without affecting platelet cyclo-oxygenase (Figure 13). Upon platelet activation of constitutively activated JAK2-platelets by shear stress starts the membrane phospholipids → phospholipase A₂ → arachidonic acid (AA) → cyclooxygenase biochemical pathway induced prostaglandin endoperoxides G₂ and H₂ production by platelets are the cause of the inflammatory signs erythromelalgia (Figure 4) featured by fibromuscular intimal proliferation and occlusive platelet thrombi (Figures 9 and 10). Release of platelet derived growth factor accounts for the fibromuscular intimal proliferation (Figures 6, 9 and 10) followed by von Willebrand (VWF) rich occlusive platelet thrombi (Figure 15). As platelets do not have a nucleus, irreversible inhibition of platelet cyclo-oxygenase (COX-1) persists for the rest of platelet life time in the circulation and cures erythromelalgia and migraine-like cerebrovascular ischemic manifestations^[37,52,53].

erythrocytes $7.6 \times 10^{12}/L$ white blood cells $14 \times 10^9/L$ and platelets $1350 \times 10^9/L$.

The spectrum of erythromelalgia complicated by painful acrocyanosis and digital gangrene has been described as the first manifestation of ET^[28-32]. Erythromelalgia patients in the study of Smith and Allen discovered that one dose of aspirin (350 to 500 mg) immediately relieved erythromelalgic pain within one hour and held on for three days, which is much longer than the analgesic effect of acetylsalicylic acid

(Figure 3)^[33]. This lasting effect of aspirin for three days due to irreversible platelet cyclooxygenase inhibition appeared pathognomonic for erythromelalgia and became the clue for the diagnosis of myeloproliferative thrombocytopenia indicating a causal relation between erythromelalgia and clonal thrombocytopenia in ET and PV patients (Figures 3 and 4)^[26,28,29,34-37]. Aspirin responsive erythromelalgia is the presenting symptom of ET at platelet counts above $400 \times 10^9/L$, but has never been observed in reactive thrombocytosis. PV when accompanied by erythromelalgia had increased platelet count indicative for associated thrombocytopenia^[37]. The complete relief (cure) of burning pain and red congestion by one dose aspirin (350 to 500 mg) for a few days is diagnostic for thrombocytopenia in ET and PV patients^[34,37]. Michiels and Van Vliet used since 1976 malondialdehyde (MDA) production in platelet rich plasma after incubation of platelet rich plasma with NEM according to Smith *et al*^[4] as an objective measure for the inhibition of cyclooxygenase and prostaglandin endoperoxide formation in thrombocytopenia platelets by which we discovered that the longlasting pain relief of erythromelalgia by aspirin (500 mg) was of similar duration as that one dose aspirin (500 mg) irreversibly inhibited platelet cyclooxygenase for a few days (Figure 3)^[37]. Reversible inhibition of platelet COX-1 activity by indomethacin 25 mg TID is an alternative to relieve erythromelalgia (Figure 3)^[37]. In contrast, sodium salicylate has no effect on platelet cyclo-oxygenase activity and did not affect erythromelalgia. Sodium salicylate but also ticlopidine (platelet ADP-receptor inhibitor) and other platelet inhibiting agents like dipyridamol did not inhibit platelet cyclo-oxygenase activity and were absolutely not effective in the treatment of erythromelalgia^[37]. Michiels and Van Vliet (1978) concluded that erythromelalgia is caused by ongoing platelet cyclooxygenase-mediated inflammatory and microvascular ischemic thrombotic processes restricted to myeloproliferative ET thrombocytopenia in patients with ET and PV. This was the start of prospective clinical, laboratory, histopathology and platelet kinetic studies initiated by Michiels to further explore the pathophysiology of Erythromelalgia in Thrombocytopenia^[37] at the Hematology Department of the Academic Hospital Dijkzigt, Erasmus University Rotterdam.

ROTTERDAM CLINICAL AND PATHOLOGIC FOR ET AND PV

Dameshek^[38] (1950), Kurnick *et al*^[39] (1972) and Michiels^[37] (1980) showed that trilinear bone marrow hypercellularity of megakaryo/erythro/granulopoiesis combined with increased erythrocytes above $6 \times 10^{12}/L$ is a pathognomonic diagnostic for PV (Table 1) and clearly differentiates between PV from primary or secondary erythrocytosis obviating the need to measure red cell mass^[37]. Bone marrow histopathology is the

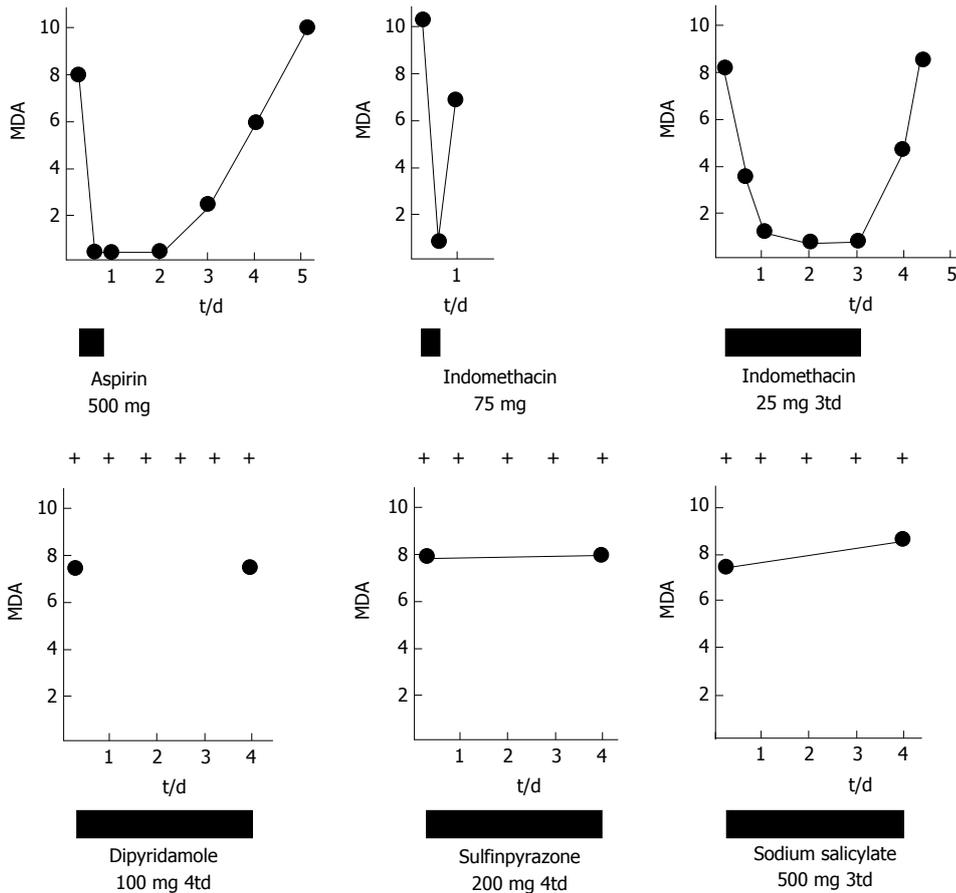


Figure 3 The effect of platelet aggregation inhibiting drugs on stimulated platelet aggregation, erythromelalgia and malondialdehyde concentration in **N-ethylmaleimide stimulated platelet rich plasma**. The effect of aspirin (acetylsalicylic acid), indomethacin, dipyridamol, sulfinpyrazon and sodium salicylate on erythromelalgia and MDA production by arachidonic acid stimulated platelets in platelet-rich plasma of symptomatic thrombocythemia patients with ET or PV complicated by erythromelalgia. MDA: Malondialdehyde.

most accurate diagnostic clue to ET and early and overt stages of PV by the demonstration of increase of clustered large and mature megakaryocytes (Table 1). Symptoms of erythromelalgia and atypical migraine-like atypical transient ischemic attacks (MIAs) in thrombocythemia patients already occurred at platelet counts above $400 \times 10^9/L$ in ET and PV patients (Table 1). Bone marrow histology shows an increase of clustered, mature, large megakaryocytes with normal or slightly increased cellularity in ET and increased cellularity due to increase of erythropoiesis in early PV (Figure 5)^[37]. Confirmative criteria for the diagnosis of ET and PV were normal erythrocyte sedimentation rate (ESR) and elevated score for leukocyte alkaline phosphatase (LAP) in the absence of infection. Bone marrow histology has the power to differentiate myeloproliferative ET from reactive thrombocytosis, from thrombocythemia in Philadelphia-chromosome positive (Ph+) ET and from 5q-minus syndrome with thrombocytosis^[37]. The megakaryocytes in Ph+ ET and in the chronic phase of Ph+ CML the megakaryocytes are smaller than normal with round nuclei showing little lobulation (Figure 5)^[40,41]. The megakaryocytes in the 5q-minus syndrome are small with dysplastic nuclei.

The minimum platelet count of $1000 \times 10^9/L$ was

required by the Polycythemia Vera study Group (PVSG, 1975) for the diagnosis of ET^[42]. In 1980 Michiels^[37] defined ET as a novel early stage MPD at platelet count between 400 and $1000 \times 10^9/L$ overlooked by the PVSG (Table 1). Wasserman^[43] (1972) and Berlin^[44] (1975) proposed a new set of major (A) and minor (B) criteria for PV patients to be included in the randomized clinical trial PVSG 01 study^[44]. The clinical PVSG criteria did not use bone marrow pathology and are crude to be sure that patients included in the PVSG 01 study indeed suffered from PV and not from secondary erythrocytosis (Wasserman personal communication)^[45]. Pearson and Whetherley-Mein showed in 1979 significant shortcomings of the 1975 PVSG criteria for PV in a prospective evaluation of 30 PV patients by the demonstration that the PVSG criteria overlook the early erythrocythemic PV cases with normal leukocytes, platelets and spleen size^[46].

SPECTRUM OF EYTHROMELALGIC THROMBOTIC THROMBOCYTHEMIA: ETT

The time lapse between the appearance of erythro-

Table 1 The 1980 Rotterdam Clinical and Pathological criteria for essential thrombocythemia and polycythemia vera

The 1980 RCP criteria for prefibrotic ET ^[37,52]	
Major criteria	
A1	Persistent platelet count in excess of $400 \times 10^9/L$
A2	Increase and clustering of enlarged megakaryocytes in bone marrow biopsy
A3	No or slight increase of reticulin fibers (RF 0 or RF 1)
Confirmative criteria	
B1	Presence of large platelets in a peripheral blood smear
B3	No or slight splenomegaly on ultrasound sonography (length diameter normal value < 12 cm)
B4	Increase of LAP-score and no signs of fever or inflammation
Exclusion criterion	
Ph+ chromosome and any other cytogenetic abnormality in blood or bone marrow nucleated cells	
The 1980 RCP criteria for prefibrotic PV to replace the crude 1975 PVSG criteria for PV	
Major	
A1	The combination of erythrocyte count of $> 6 \times 10^{12}/L$ and bone marrow hypercellularity due to EM or EMG hyperproliferation is pathognomonic diagnostic for PV (Dameshek and Hentzel ^[27] 1940, Dameshek ^[38] 1950, Kurnike <i>et al</i> ^[39] 1972) obviating the need to measure raised red cell mass
A2	Increase in bone marrow biopsy of clustered, enlarged pleomorphic megakaryocytes with hyperlobulated nuclei and moderate to marked increase cellularity of megakaryopoiesis/erythropoiesis or typically trilinear mega-erythro-granulopoiesis (EMG). Such a typical PV bone marrow picture excludes all variant of primary and secondary erythrocytosis ^[37-39]
Minor	
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 score > 100 , absence of fever or infection
B4	Splenomegaly on ultrasound sonography
A1 + A2 establish PV and exclude erythrocytosis. One or more of B confirm PV	

RCP: Rotterdam Clinical and Pathological; ET: Essential thrombocythemia; PV: Polycythemia vera; EM: Erythrocythic megakaryocytic; EMG: Erythrocytic megakaryocytic granulocytic.



Figure 4 Isothermgrams of two essential thrombocythemia patients with erythromelalgia in toes and fore foot sole. Typical mottled red blue congestion and thermographic visualization of erythromelalgia in the fore foot and toes. Skin surface temperature: blue 24 °C-25 °C; green 26 °C-27 °C; purple 28 °C-29 °C; red 30 °C-31 °C; yellow 32 °C and white 33 °C. Complete correction of the upper leg thermograms after effective treatment with aspirin.

Table 2 Localization of erythromelalgia in feet/toes vs fingers and skin, and the presence of peripheral gangrene and history of acute coronary syndrome or migraine-like cerebral ischemic attacks, and time lap between first manifestations of erythromelalgia and diagnosis of thrombocythemia in essential thrombocythemia (*n* = 11) and polycythemia vera (*n* = 13)

Patient	Diagnosis	Feet toes	Fingers	Skin	PG	ACS	MIAs	Time lap (mo)
1	ET	Bilateral		Present	Yes	Yes	Yes	45
2	ET	Bilateral			Yes		Yes	154
3	ET							60
4	ET	Unilateral			Yes	Yes		12
5	ET	Unilateral	Unilateral	Present	Yes			4
6	ET					Yes	Yes	20
7	ET						Yes	60
8	ET							30
9	ET	Bilateral			Yes			20
10	ET	Bilateral	Bilateral	Present	Yes			30
11	ET	Bilateral		Present				30
12	PV	Unilateral						24
13	PV	Unilateral						3
14	PV	Bilateral						0
15	PV		Unilateral	Present	Yes	Yes		36
16	PV	Bilateral					Yes	48
17	PV	Unilateral						1
18	PV	Bilateral					Yes	18
19	PV		Bilateral					2
20	PV	Bilateral			Yes			24
21	PV	Unilateral						4
22	PV	Unilateral		Present				3
23	PV	Unilateral	Unilateral					24
24	PV		Unilateral					6

PG: Peripheral gangrene; ACS: Acute coronary syndrome; ET: Essential thrombocythemia; PV: Polycythemia vera; MIAs: Migraine-like cerebral ischemic attacks.

melalgic symptoms and diagnosis of thrombocythemia in my first cohort of 24 patients with erythromelalgic thrombotic thrombocythemia (ETT: 11 ET and 13 PV) ranged from a few months in eight, and from 1 to 5 years in 15 cases due to the lack of knowledge of a causal relation between erythromelalgia and thrombocythemia^[37,47]. The lowest platelet count in ET at which erythromelalgia occurred was around $400 \times 10^9/L$ ^[47]. Twenty four ETT (11 ET and 13 PV) patients, presented with erythromelalgia complicated by microvascular disturbances including peripheral acrocyanosis or gangrene (thromboangiitis obliterans) in 8, acute coronary syndrome in 4 and transient neurologic ischemic attacks in 6 (Table 2). Erythromelalgia was localized in toes and foot soles in 17, in fingers in 8 and the skin of lower or upper legs in 6 (Table 2)^[37]. Localization of erythromelalgia in the skin in 6 thrombocythemia patients (4 ET and 2 PV) was misdiagnosed as superficial thrombophlebitis (Table 2 and Figure 6). Thermographic measuring of the skin surface temperature using a Bofors Mark II camera showed that the burning pain and red congestion of erythromelalgia started to occur when the skin surface temperature exceeded the critical level of 31°C, and ameliorated to disabling suffer at increasing skin temperature above 31 °C (Figures 4 and 6)^[37]. This is in accordance with observation of Brown^[48] (1932) and Smith and Allen^[33].

PLATELET KINETIC STUDIES IN THROMBOCYTHEMIA COMPLICATED BY ERYTHROMELALGIA

Platelet kinetic investigations according to Branehög^[49,50] was used to document the involvement of platelets in the etiology of erythromelalgia in thrombocythemia. Platelet kinetic studies were performed in 4 control persons, 6 asymptomatic thrombocytosis (3 with reactive thrombocytosis: RT and 3 chronic myeloid leukemia: CML), 6 asymptomatic thrombocythemia patients, and 8 thrombocythemia patients complicated by erythromelalgia (Figure 7 and Table 3)^[37]. Measuring of half life times (T1/2, mean survival (MS) and maximal life times (MaxLS) of ⁵¹Cr labeled autologous platelets in the circulation appeared to be a trustful objective method to demonstrate platelet consumption in ongoing thrombotic processes (Figure 7)^[37]. MS and MaxLS are equal in T (reactive thrombocytosis) and in E- indicating that the Cr platelet disappearance curves are linear or near to linear indicating a normal platelet survival with a T1/2 of around 4 d (Figure 7). In E+ MS is significantly shorter than MaxLS indicating a shortened Cr platelet survival with a T1/2 of around 2 d with curvilinear platelet disappearance curves (Figure 7).

Aspirin treatment of 7 symptomatic thrombocythemia resulted in the disappearance of erythromelalgia, significant increase of peripheral blood platelet counts,

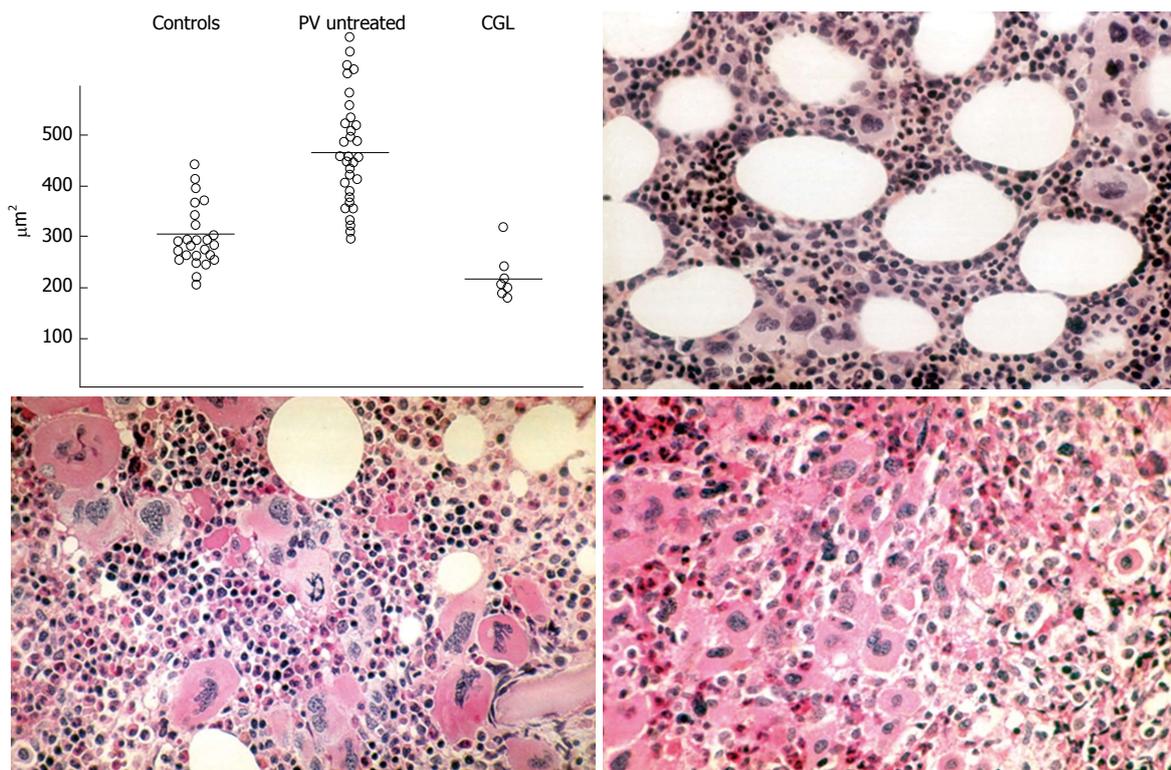


Figure 5 Planimetry of megakaryocyte sizes (μm^2) from bone marrow smears in controls, polycythemia vera and chronic granulocytic leukemia upper left: Normal size megakaryocytes in controls; large megakaryocytes in untreated polycythemia vera and small sized megakaryocytes in chronic granulocytic leukemia (Frantzen *et al*^[40]). Demonstration by Michiels (1981) of a spectrum of clustered large megakaryocytes with hyperlobulated nuclei and a normocellular bone marrow in essential thrombocythemia (ET) vs increased bone marrow cellularity due to increased erythropoiesis in ET and polycythemia vera (PV) vs increased trilinear erythrocytic, megakaryocytic and granulocytic (EMG) proliferation in classical PV according to Dameshek^[38] (1950) and Kurnicke *et al*^[39].

correction of platelet survival times and platelet disappearance curves to normal (Figure 8)^[37]. These data on platelet consumption and its correction by aspirin in symptomatic thrombocythemia patients demonstrate that erythromelalgic thrombotic complications of thrombocythemia including transient cerebral and ocular ischemic attacks are caused by spontaneous activation of hypersensitive platelets at high shear in the arteriolar endarterial circulation as first documented by Michiels and Ten Kate in skin biopsies (Figures 9 and 10)^[37,51,52]. The platelet-mediated erythromelalgic microvascular thrombotic complications are cured by aspirin and platelet reduction to normal ($< 400 \times 10^9/\text{L}$), but not by coumadin and not by the ADP inhibitor ticlopidin^[37,52]. Erythromelalgic thrombotic complications in thrombocythemia associated with Ph+ ET or CML is rare^[37]. Despite the high platelet counts, patients with reactive thrombocytosis and thrombocytosis in Ph+ CML patients do not present with erythromelalgic microvascular ischemic events. In Ph+ thrombocythemia, the platelets are small, indolent and non-reactive, whereas the platelets in thrombocythemia of ET and PV patients are large and hypersensitive with clinical evidence of platelet-mediated erythromelalgic thrombotic manifestations^[37,41].

HISTOPATHOLOGY OF ERYTHROMELALGIA IN THROMBOCYTHEMIA

As there were no reports in the 1970s on the histopatho-

logical substrate of aspirin responsive erythromelalgia, Michiels and Ten Kate performed in 1984 skin punch biopsies for histopathological investigations in ET patients from recently relapsed red congested erythromelalgia in the fore foot sole within one week after discontinuation (Figure 9)^[37,51,52]. The arterioles in the deep reticular dermis show strong proliferation and degenerative vessel wall changes and the venules, capillaries and nerves are not involved (Figure 9). The zone of proliferated cells in the intima is two to three layers thick and distinct from the smooth muscle cells of the media. Immunofluorescence studies using antibodies against FVIII and smooth muscle cells revealed that the intimal proliferation was caused by proliferation by smooth muscle cells covered by one layer of endothelial cells (Figure 9). The endothelial cells (EC) are swollen and have large nuclei indicative for activated ECs (Figure 9). The venules, capillaries and nerves were not involved^[37,51]. The membrana elastica interna (mei) at places of fibromuscular intimal proliferation is broken up and splitted by the proliferating smooth muscle cells (Figure 9)^[37,51]. Histopathology of skin punch biopsies from relapsed acrocyanotic erythromelalgia three weeks after discontinuation of aspirin are featured by fresh thrombotic occlusion on top of fibromuscular intimal proliferation in arterioles, whereas the venules, capillaries and nerves were not involved (Figure 10)^[37,51,52]. The histopathology of longstanding untreated erythromelalgia complicated by digital gangrene mainly

Table 3 Results of ⁵¹Cr autologous platelet survival studies in 4 controls (Group I), in 3 cases of thrombocytosis in chronic myeloid leukemia and 3 cases of reactive thrombocytosis (Group II), in 6 cases of asymptomatic thrombocythemia in essential thrombocythemia, myelofibrosis and polycythemia vera (Group III), and in 8 cases of thrombocytosis in essential thrombocythemia, myelofibrosis and polycythemia vera complicated by erythromelalgia (Group IV)

Patient group	Diagnosis	Platelet, × 10 ⁹ /L	E	T1/2 (d)	Mean life span	Maximal life span
I		210	No	3.6	5.4	9.9
		181	No	4.2	9.0	9.1
		193	No	3.9	7.1	7.8
		138	No	3.7	6.0	8.8
Mean		180		3.9	6.9	8.9
II		722	CML No	4.0	8.6	8.2
		1487	CML No	3.9	7.6	7.3
		2244	CML No	4.0	7.4	7.7
		1015	RT No	4.0	6.7	8.7
		736	RT No	4.0	6.6	7.8
		866	RT No	4.9	9.7	9.2
Mean		1178		4.1	7.9	8.2
III		1722	ET No	3.4	5.9	6.8
		1167	ET No	3.0	4.6	7.3
		511	MF No	3.1	4.5	8.8
		935	PV No	3.8	6.2	9.0
		506	PV No	3.5	5.8	8.8
		614	PV No	3.3	5.7	7.5
Mean		918		3.3	5.4	8.0
IV		666	ET Yes	2.1	2.9	6.4
		637	ET Yes	2.6	4.0	6.8
		1018	ET Yes	2.7	4.2	7.2
		539	MF Yes	1.8	2.6	6.1
		489	PV Yes	2.7	4.0	7.9
		1028	PV Yes	2.5	1.7	7.3
		1036	PV Yes	2.0	3.4	5.6
		1180	PV Yes	3.1	6.0	5.8
Mean		824		2.4	3.6	6.6

CML: Chronic myeloid leukemia; RT: Reactive thrombocytosis; ET: Essential thrombocythemia; PV: Polycythemia vera; MF: Myelofibrosis.

show completely occluded arterioles by fibrotic organized thrombi (Figure 10). If overlooked and not treated with aspirin endstage erythromelalgia result in painful acrocyanotic cold toes and fore foot showing onion-like structures of occluded arterioles due to vascular and perivascular fibrosis (Figure 10)^[37,51,52].

ERYTHROMELALGIA CURED BY ASPIRIN AND CORRECTION OF PLATELET NUMBER TO NORMAL

Twenty three patients with erythromelalgia (Figure 11) were treated with aspirin between 1974 and 1985)^[37,52]. The cure of erythromelalgia by aspirin could be documented in 15 thrombocythemia patients. Some erythromelalgic thrombocythemia patients had already themselves discovered the favorable effect of aspirin on erythromelalgia. Remission of thrombocythemia by busulfan was defined by reduction of platelet count to below 350 × 10⁹/L, and relapse by increase of

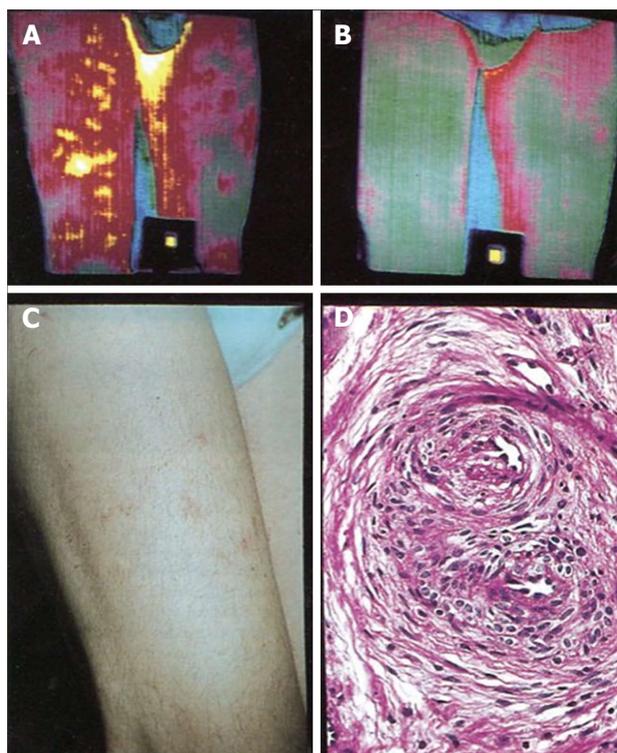


Figure 6 Isothermograms of upper legs showing “superficial thrombophlebitis” (A) in the right upper leg, which completely disappeared after treatment with aspirin once daily (B) and superficial thrombophlebitis manifested as red painful indurated hot spots, erythromelalgia of the skin (C) in the upper leg caused by fibromuscular intimal proliferation (endarteritis obliterans) as documented by histology from skin punch biopsies (D) from the red spots.

platelet count above 400 × 10⁹/L. The erythromelalgia disappeared completely after reduction of platelet count to less than 400 × 10⁹/L and did not re-appear after discontinuation of aspirin at platelet count below 400 × 10⁹/L^[37,52]. Aspirin was discontinued in busulfan induced thrombocythemia with normal platelet count in 13 ET and 11 PV patients (Figure 11)^[37,52]. Erythromelalgia recurred in 8 of 12 patients (9 ET and 2 PV) already at platelet counts between 400 to 550 × 10⁹/L (Figure 11). Remission duration of thrombocythemia (platelet 400 to 500 × 10⁹/L) by one course busulfan lasted from 2 to more than 9 years (long busulfan remitters), which was associated with the disappearances of erythromelalgia and with no reappearance of erythromelalgia after discontinuation of aspirin in ET patients 1 to 6 and 9 and 10 (Figure 11)^[52]. Phlebotomy in PV did not improve erythromelalgia in PV. Patients 16, 19, 20 and 26 with PV received long-term aspirin therapy, which gave complete symptomatic relief of erythromelalgia and cure circulatory disturbances for the duration of aspirin administration at increased platelet counts (Figure 11). Busulphan induced normal platelet count was reached in all PV patients, but the remission duration of erythromelalgic thrombocythemia was much shorter (short busulfan remitters) as compared to ET^[52]. The final follow-up of the effect of one course busulfan in 20

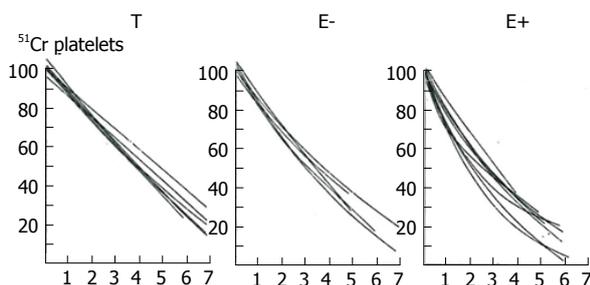
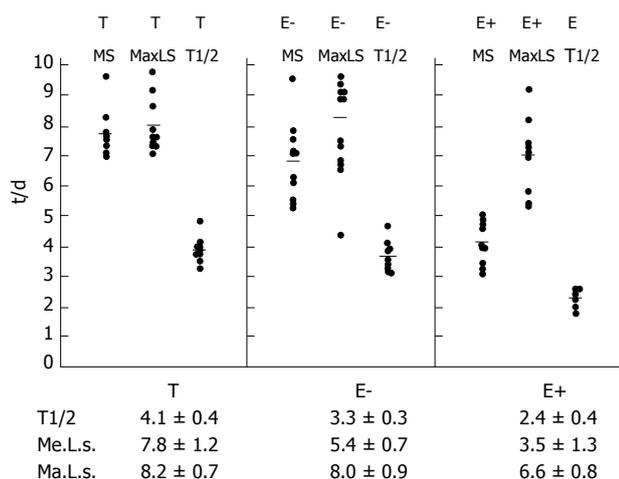


Figure 7 Platelet survival times and platelet disappearance curves according to Branehög *et al*^[50] in 10 thrombocytosis patients complicated by erythromelalgia (E+), 10 asymptomatic thrombocytosis patients (E-), and 11 asymptomatic patients with thrombocytosis (T). Curvilinear platelet survival curves in E+ indicates a consumptive disappearance of thrombocytosis platelets from the circulation; Slight curvilinear platelet survival curves in E- suggest slight, but insignificant random platelet consumption; and linear platelet survival curves in group T with reactive thrombocytosis indicate a non-random, age-related disappearance of platelets from the circulation. MS = mean survival. T1/2; MaLS = maximal life span according to Branehög *et al*^[50].

symptomatic ET in the period between 1974 to 1986 has been reported in 1999 in great detail^[47].

PATHOPHYSIOLOGY OF ASPIRIN RESPONSIVE PLATELET MEDIATED ERYTHROMELALGIA

Spontaneous activation and aggregation at high shear in arterioles of JAK2 constitutively activated (sticky) platelets induce high levels of arachidonic acid (AA) release from platelet membrane phospholipids with the subsequent transition of AA cyclooxygenase in to large amount of prostaglandin endoperoxides followed by the generation of thromboxane A2 (Figure 1) appear to be of critical importance for the inflammatory signs, fibromuscular intimal proliferation and platelet thrombi in JAK2^{V617F} mutated thrombocytosis (Figure 12). In this process secondary activation of platelets by ADP (P2Y12), thrombin or collagen receptor mediated aggregation does not play any role, thereby explaining the ineffectiveness of ticlopidin and clopidogrel in the treatment of erythromelalgia

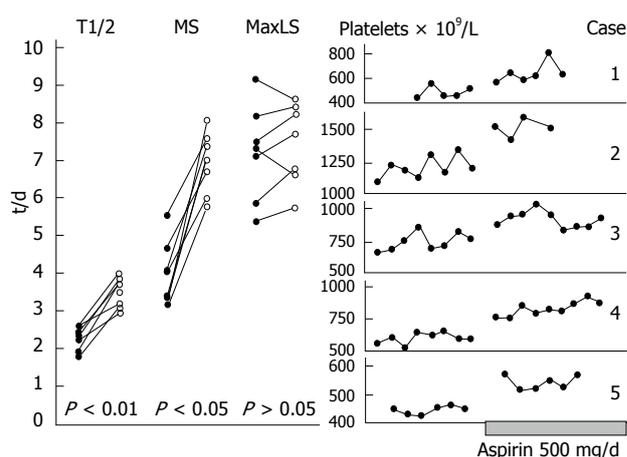


Figure 8 Platelet survival curves in seven E+ thrombocytosis patients before and after aspirin 500 mg/day, and peripheral blood platelet counts before and after maintenance aspirin treatment 250 mg/d. MS: Mean survival; T1/2; MaLS: Maximal life span according to Branehög *et al*^[50].

(Figure 13). This novel insight has very important clinical implications in our current understanding that spontaneous activation of hypersensitive JAK2^{V617F} mutated thrombocytosis platelets at high shear in arterioles causes erythromelalgia due to the release of large amounts of prostaglandin endoperoxides and thromboxane A2, that can explain both the pronounced inflammatory, fibromuscular intimal proliferation and thrombosis in arterioles. The cure of erythromelalgia by aspirin is due to complete inhibition of prostaglandin endoperoxide (PGE2) and thromboxane A2 through irreversible inhibition of platelet cyclooxygenase (Figures 1, 3, 12 and 13). Aspirin indeed became a wonder drug that cured platelet mediated erythromelalgia in myeloproliferative JAK2^{V617F} mutated thrombocytosis in ET and PV patients by irreversible inhibition of platelet cyclooxygenase^[5,37,51,52] (Figures 3, 12 and 13). The novel key observation in this report anno 2017 is that spontaneous activation and aggregation of hypersensitive JAK2^{V617F}-mutated sticky platelets is associated with the generation of large amounts of AA induced cyclic endoperoxides including PGE2 and thromboxane A2 as compared to ADP (P2Y12) induced aggregation by ticlopidin and clopidogrel (Figures 3, 12, 13). This lucid insight can fully explain the occurrence of the inflammatory manifestations of erythromelalgia caused by shear stress induced activation of hypersensitive platelets in thrombocytosis as the first stage of red congested erythromelalgia (Figures 3, 12 and 14) followed by fibromuscular intimal proliferation in skin areas of erythromelalgia (Figure 9). If not treated with aspirin, occlusion by von Willebrand factor (VWF) platelet rich thrombi occur at places of vessel wall damage of fibromuscular intimal proliferation (Figure 15)^[51-53]. Coumadin and the platelet ADP (P2Y12) inhibitors ticlopidin and clopidogrel are ineffective. Treatment with a loading dose 350 to 500 mg followed by 100 mg once daily cures erythromelalgia, its acrocyanotic complications

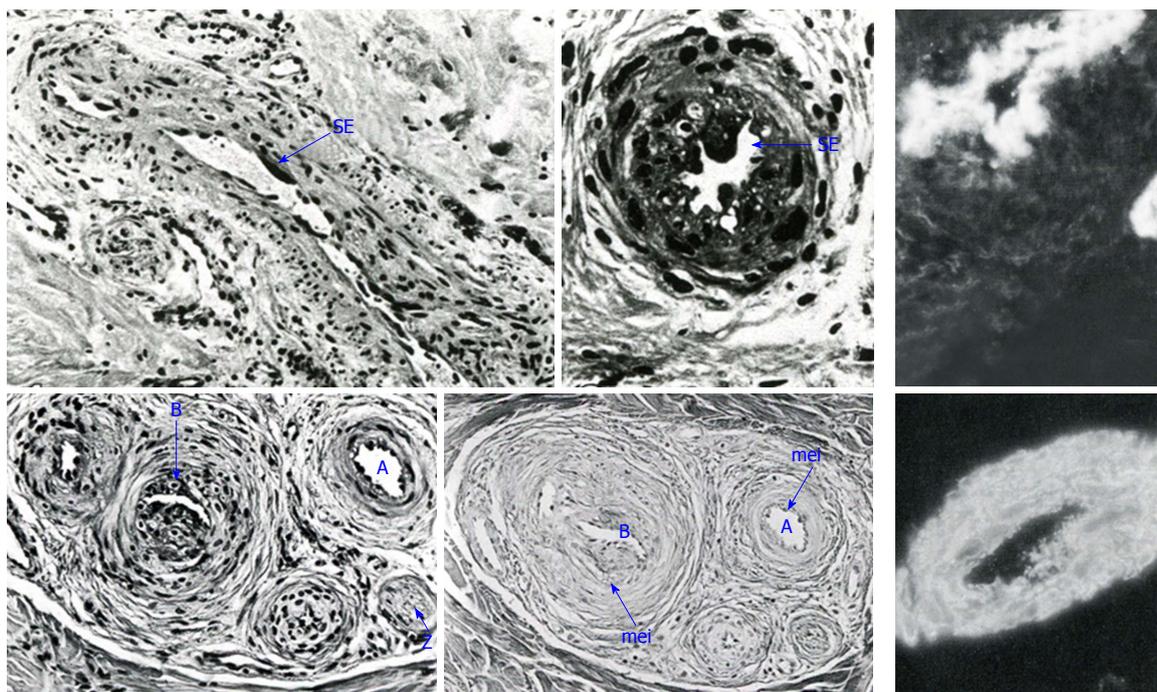


Figure 9 Arterioles with swelling of endothelial cells, proliferation of cells of the inner layer below the media and normal venules, capillaries and nerves (upper and left), and elastica von Gieson stain showing a normal membrana elastica interna (mei) in a normal arteriole (A). Source Michiels 1981. The membrana elastica interna (mei) is splitted up and fragmented between the proliferating cells in arteriole B with intimal proliferation in in skin areas of very typical red congested erythromelalgia within one week after discontinuation of aspirin in two cases with essential thrombocythemia. Source Michiels 1981. Immunofluorescence of proliferating cells in the intima of affected arterioles shows on layer endothelial cells with antiserum against factor VIII and multilayer proliferation of smooth muscle cells with antiserum against smooth muscle cells indicative for fibromuscular intimal proliferation of affected arterioles in erythromelalgic thrombocythemia (upper). Partial and complete occlusion by a fresh thrombus in acrocyanotic erthomelalgia three weeks after discontinuation of aspirin (lower). Source Michiels 1981.

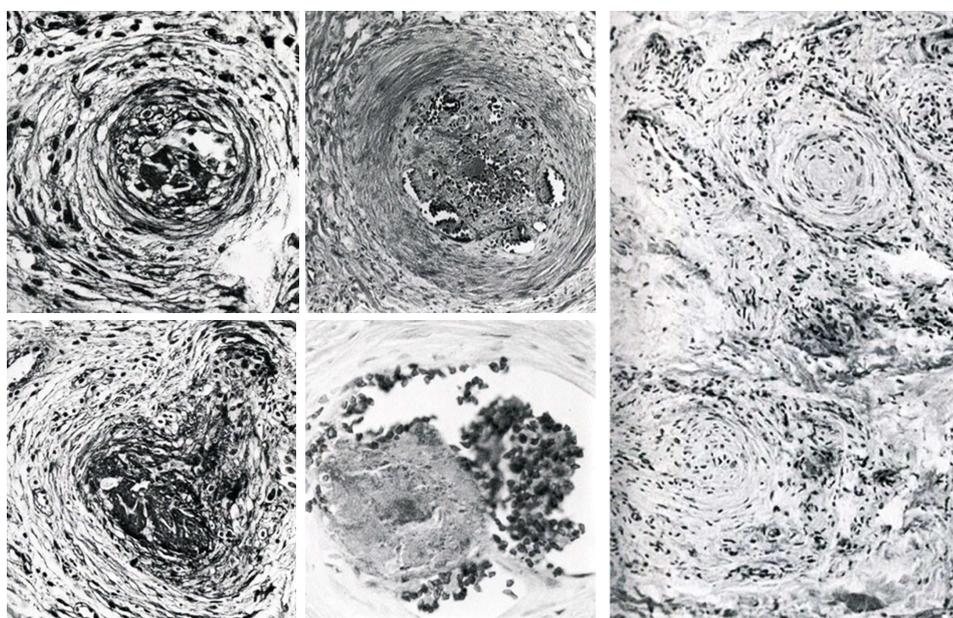


Figure 10 Thrombotic occlusion of arterioles on top of fibrousular intimal proliferation. Thromboangiitis obliterans (left panel) and recanalisation of arterioles showing vessel wall fibrosis of arterioles in two cases of erythromelalgia complicated by acrocyanosis and digital gangrene (middle panel). Source Michiels 1981. Ony structure by vascular and perivascular fibrosis of occluded fibromuscular intimal proliferation in acrocyanotic digital ischemia of untreated endstage erythromelalgia that had transformed into aspirin resistant Raynaud phenomenon (right). Source Michiels 1981.

as well as the migraine-like atypical TIAs (MIAs) and acute coronary syndromes (ACS) through irreversible inhibition of platelet cyclo-oxygenase (Figures 13-15).

The cure of erythromelalgia by aspirin OD could be attributed to maintained irreversible inhibition of platelet cyclo-oxygenase keeping the prostacycline cyclo-

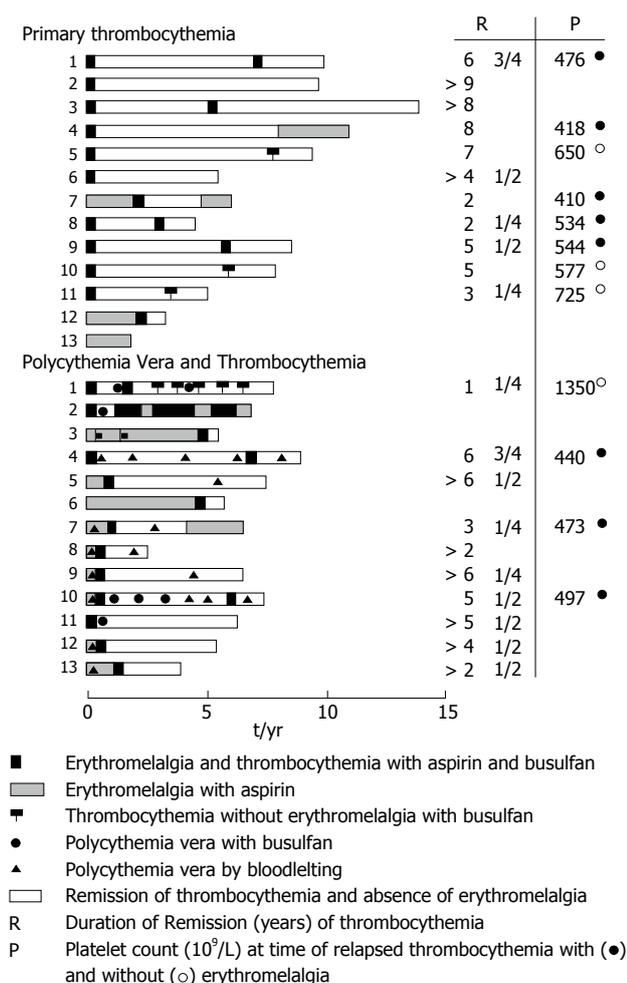


Figure 11 Results of treatment in 13 patients with primary or essential thrombocythemia and 13 patients with polycythemia vera and thrombocythemia. Source Michiels *et al*^[53], 1985. Busulfan induced remission of thrombocythemia (platelet counts < 350 × 10⁹/L) lasted 2 to 9 years (long busulfan remitters) in essential thrombocythemia (ET) patients, which became asymptomatic with no recurrence of erythromelalgia after discontinuation of aspirin during thrombocythemia remission periods of 2 to 9 years (R). Erythromelalgia recurred in eight [5 ET, 3 polycythemia vera (PV)] of twelve (8ET, 4PV) at platelet counts between 400 to 550 × 10⁹/L (P) after thrombocythemia remission periods of 2 to 8 years. Busulfan induced remissions of thrombocythemia in the majority of PV patients lasted several months to a few years (short busulfan remitters) indicating the need to treat with repeated courses of busulfan.

oxygenase in endothelial cells intact (Figures 2 and 15)^[37,52,53]. The platelet inhibiting drugs sulfipyrazone, dipryridamole and ticlopedine do not inhibit platelet cyco-oxygenase activity and had no effect on erythromelalgia (Figures 3 and 15)^[37,53]. Spontaneous activation, aggregation, secretion of JAK2 constitutively activated, hypersensitive sticky platelets became the key cause in the etiopathogenesis of erythromelalgia, MIAs and ACS in JAK2-mutated thrombocythemia (Figures 14 and 15). PDGF in this process accounts for the fibromuscular intimal proliferation^[20,21]. Vaso-active substances, prostaglandins endoperoxides and other factors released during JAK2^{V617F} mutated platelet aggregation account for the inflammatory symptoms

(Figure 15)^[52,53]. Platelet kinetic studies demonstrated that in the presence of erythromelalgia platelet consumption is increased as the final proof of platelet cyclo-oxygenase mediated etiology of erythromelalgic inflammatory and arteriolar (end-arterial) microvascular thrombosis in JAK2^{V617F}-mutated thrombocythemia in ET and PV patients (Figures 7 and 8)^[37]. Biopsies from erythromelalgic areas in five ET patients show arteriolar lesions of fibromuscular intimal proliferation without involvement of venules, capillaries and nerves (Figure 9)^[37,51]. If left untreated erythromelalgia leads to ischemic symptoms of acrocyanosis and peripheral gangrene due to thromboangiitic occlusions of arterioles on top of platelet cyclooxygenase mediated fibromuscular intimal proliferation (Figure 10)^[37].

CLINICAL MANIFESTATIONS OF PV: THERAPEUTIC IMPLICATIONS

The presenting clinical manifestations in PV patients include microvascular events, ranging from erythromelalgic ischemia of a toe or finger, amputation of one or more gangrenous digits (thrombo-angiitis obliterans), attacks of transient blindness (amaurosis fugax), MIAs, facial weakness or aphasia, superficial thrombophlebitis and major thrombosis including stroke, coronary artery disease, deep vein thrombosis, splanchnic vein thrombosis and pulmonary embolism^[54-59]. The intrinsic blood changes in PV as a trilinear MPN (Table 1)^[37-39] are increased platelets, erythrocytes, hematocrit, activated leukocytes and blood cellular viscosity, which are responsible for this altered distribution of minor and major vascular complications in PV as compared to the high incidence of microvascular and low incidence of major thrombotic manifestations in the rotterdam clinical and pathologic (RCP) defined ET of the Dutch Collaborative Low-dose Aspirin in ET (Dutch CLAT) studies^[37,52,60-70]. Low-dose aspirin in ET and combined aspirin and phlebotomy in PV are highly effective in the reduction of erythromelalgia, and microvascular ischemic disturbances in ET and PV, but partially reduce major thrombosis in PV, and do not influence the natural history of the JAK2 mutated trilinear myeloproliferative neoplasms (MPNs) in terms of leukocytosis, erythrocytosis, splenomegaly and myelofibrosis. On top of the erythromelalgic thrombotic microvascular disease of thrombocythemia (ETT) the high incidence of major thrombotic events in PV was related to high blood hematocrits due to increased erythrocyte counts above 6 × 10¹²/L (Table 1^[37-39] and Figure 12^[54-60]). In PV phlebotomy reduces the incidence of major arterial and venous thrombosis but does not improve the aspirin responsive erythromelalgia, acrocyanotic digital complications, and migraine-like atypical transient cerebral and ocular attacks (MIAs) (Figure 15 and 16). The lowest incidence of major thrombosis has been found in PV treated to achieve adequate control hematocrit to around 0.40^[58,59], but

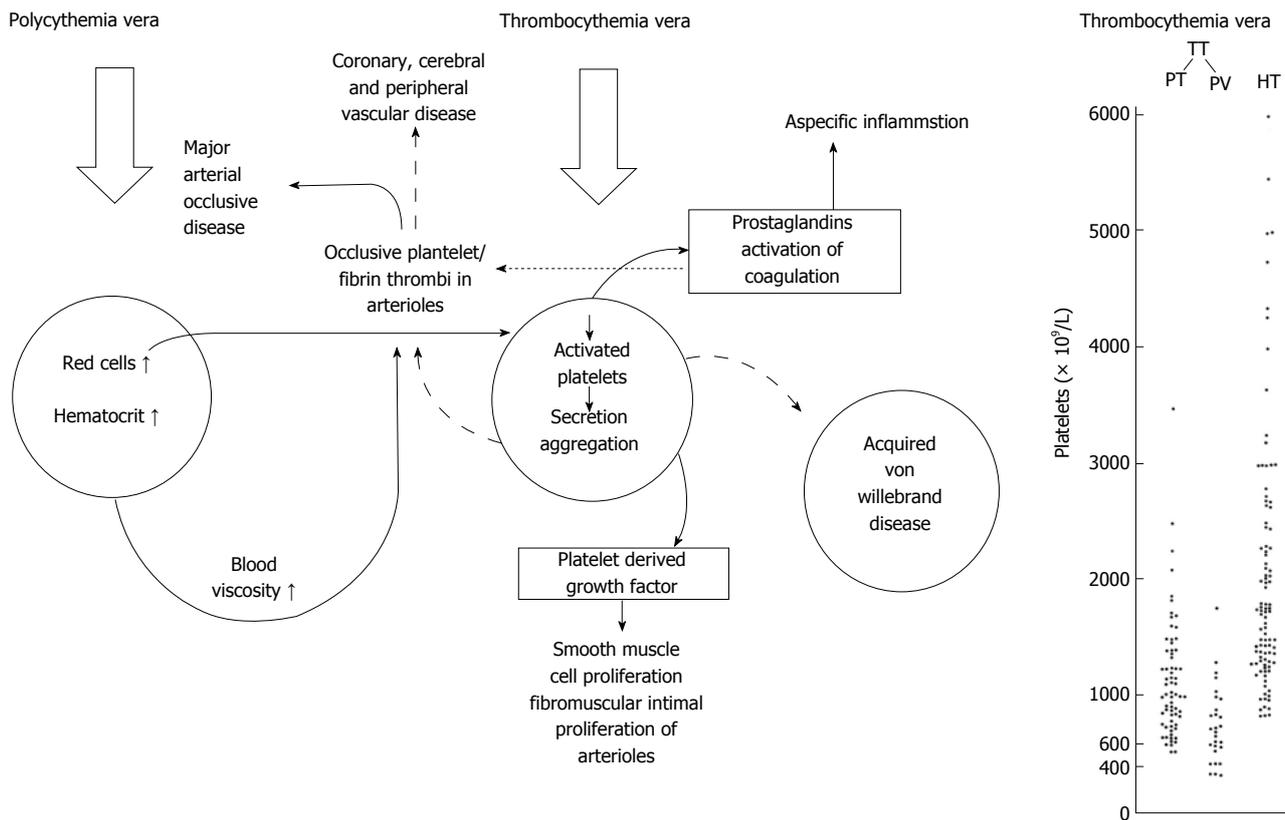
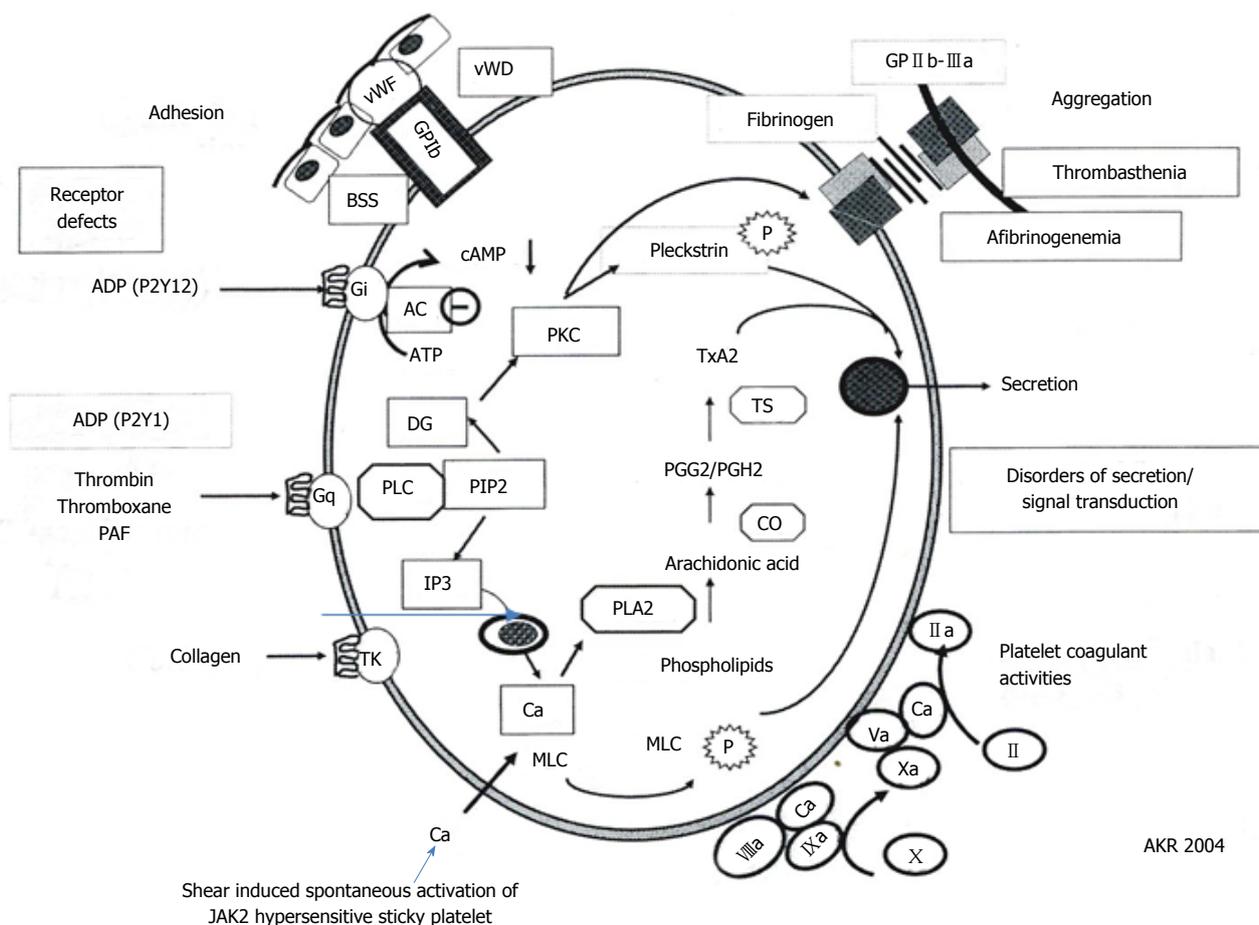


Figure 12 Pathophysiology of erythromelalgia as multicellular processes caused by platelet mediated microvascular erythromelalgic arteriolar inflammation and thrombosis in myeloproliferative thrombocytopenia vera and major arterial thrombotic disease in polycythemia vera. Shear induced production of prostaglandin endoperoxides from activated platelets in arterioles account for red warm congested swelling. Platelet derived growth factor (PDGF) released during platelet secretion can readily explain the fibromuscular intimal proliferation of arterioles first described by Michiels in 1981 and published by Michiels *et al*^[52,53] in 1984 and 1985. Right: Platelet counts in 99 case histories of erythromelalgia thrombotic thrombocytopenia (ETT) subdivided in ET ($n = 69$) and PV ($n = 30$) and in 100 case histories with hemorrhagic thrombocytopenia (HT), Source Michiels 1981.

the microvascular erythromelalgic occlusive syndrome of thrombocytopenia at platelet counts above $400 \times 10^9/L$ persists in PV in remission by phlebotomy^[58,59]. Weitherley-Mein and Michiels discussed their common experiences on microvascular disturbance, major thrombosis and bleeding in myeloproliferative ET and PV^[53-59] and strongly recommended since 1985 the use low dose aspirin for the treatment and prevention of erythromelalgic cerebral, ocular and coronary ischemic attacks in ET and PV patients in the United Kingdom and The Netherlands^[59-61]. Cure of erythromelalgia, microvascular ischemic disturbances preceding PV or in the early stages of PV patients in complete remission by phlebotomy are obtained with aspirin 40 to 50 mg OD on top of keeping the hematocrit around 0.40 in males and females at platelet between 400 to $1000 \times 10^9/L$ ^[53,55,58,69,70]. It became evident that the JAK2^{V617F} mutated platelets in trilinear MPN are large and hypersensitive (sticky) in patients carrying the JAK2^{V617F} in ET and PV. Platelet in MPL⁵¹⁵ mutated ET^[71] are also constitutively activated and hyperreactive (sticky). This novel insight can easily explain the high risk (about 40% to 60%) of platelet-mediated erythromelalgic microvascular ischemic attacks in JAK2^{V617F} ET and PV and in MPL and CALR (calreticulin) mutated ET patients without features of PV patients^[71]. If not treated with

aspirin as was the case in the Vannucchi study^[71], the incidence of major thrombosis at diagnosis and during follow-up in JAK2^{V617F} mutated ET and PV was high, but less frequent in JAK2 negative ET and PV patients^[71].

In the Dutch ET/PV studies two third of PV patients were on aspirin/phlebotomy alone and only one third needed hydroxyurea and 16% used IFN, whereas two third of PV patients treated according to the WHO recommendations were on maintained hydroxyurea treatment^[72-80]. In the Netherlands low dose pegylated interferon (IFN) became the first line treatment option in symptomatic PV with leukocytosis and mild splenomegaly to postpone the use of the leukemogenic agent hydroxyurea during long-term or even life long follow-up^[78,79]. In the ECLAP (European Collaboration on Low-dose Aspirin in PV) study^[72-74] treatment modalities at time of randomization into aspirin vs placebo were: Hydroxyurea in 44%, busulphan in 1%, pipobroman in 5.4%, IFN in 4.2% and phlebotomy alone 28%, or as adjuvant in 72%. There were no differences of vascular risk factors (like hypertension, diabetes, hyperlipidemia, previous thrombosis, etc.) in the aspirin and the placebo group. Mean values in randomized treated PV patients were 0.45 for hematocrit and $330 \times 10^9/L$ for platelet count. In this setting treatment with aspirin (100 mg OD) vs placebo



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Figure 13 A schematic representation of platelet adhesion through von Willebrand factor and glycoprotein Ib disturbances in Bernard Soulier syndrome, von Willebrand disease and aggregation disturbances in Glanzmann’s thrombasthenia, and receptor defects of ADP (P2Y12, P2Y1), thrombin, thromboxane, platelet activating factor (PAF and collagen induced intracellular metabolites). Source: Rao *et al.* Inherited defects in platelet signaling. *Semin Thromb Hemostas* 2004; 30: 525-535. Shear induced platelet activation of JAK2 hypersensitive (sticky) platelets (blue arrow) in arterioles starts with liberation of arachidonic acid from platelet membrane phospholipids by phospholipase A2 (PLA2) and metabolism of arachidonic acid by cyclooxygenase (CO) into prostaglandin endoperoxides PGG2/PGH2 and subsequent thromboxane A2 (Tx) generation by thromboxane synthetase (TS). Spontaneous activation and aggregation of JAK2 sticky platelets (arrow) occur in the arteriolar peripheral and cerebral endarterial circulation with the production of large amount of prostaglandin endoperoxides as the cause of aspirin responsive inflammatory component of erythromelalgia followed by fibromuscular intimal proliferation and occlusion platelet thrombi in arterioles (Figures 9 and 10). Aspirin cures erythromelalgia in JAK2 thrombocythemia by irreversible inhibition of platelet cyclooxygenase (CO)^[37]. In this process the activation of platelets by ADP (P2Y12), thrombin and collagen receptors are not involved in the etiology of erythromelalgia and cerebral vascular disturbances^[52,53,79,80]. vWF: Von Willebrand factor; BSS: Bernard Soulier syndrome; vWD: Von Willebrand disease; Ca: Calcium; MLC: Myosin light chain; IP3: Inositoltriphosphate; PLC: Phospholipase C; PIP2: Phosphatidylinositol biphosphate; DG: Diacylglycerol; AC: Adenylcyclase; PKC: Protein kinase C.

in the ECLAP study significantly reduced the overall risk of a combined end point of microvascular and major vascular complications, including cardiac death, non-fatal myocardial infarction and stroke and major venous thrombosis from 15.5% to 6.7% during 2.7 years follow-up^[73]. Absolute risk reduction was 8.4% and the number needed to treat to prevent one thrombotic event is 12^[71,72]. Subsequently low dose aspirin was added for prevention of vascular events in PV. Treatment of PV anno 2017 include low dose aspirin, phlebotomy, low dose IFN, hydroxyurea in combination with phlebotomy and or ruxolitinib to reduce the dose and duration of the leukemogenic agent hydroxyurea (Figure 17)^[53,78,79]. Recent insights indicate the IFN reduces both platelet and leukocyte count to normal obviating the need of aspirin and hydroxyurea life long in JAK2^{V617F}-mutated prodromal PV, classical PV and hypercellular ET with PV features (masked PV) or

without PV features in CALR thrombocythemia^[78-80] (personal experiences, manuscript in preparation).

MOLECULAR ETIOLOGY OF STICKY PLATELETS IN JAK2-THROMBOCYTHEMIA OF ET AND PV PATIENTS

With the advent of the JAK2^{V617F} mutation as the cause of trilinear myeloproliferative neoplasms (MPNs) ET and prodromal, classical and advanced PV, it became clear that JAK2^{V617F} mutated megakaryocytes are constitutively activated and do produce JAK2^{V617F} positive hypersensitive “sticky platelets” which spontaneously activate at high shear in the peripheral, ocular, cerebral and coronary endarteriolar circulation as the cause of platelet mediated arteriolar inflammation (platelet thrombophilia) in JAK2-mutated thrombocythemia

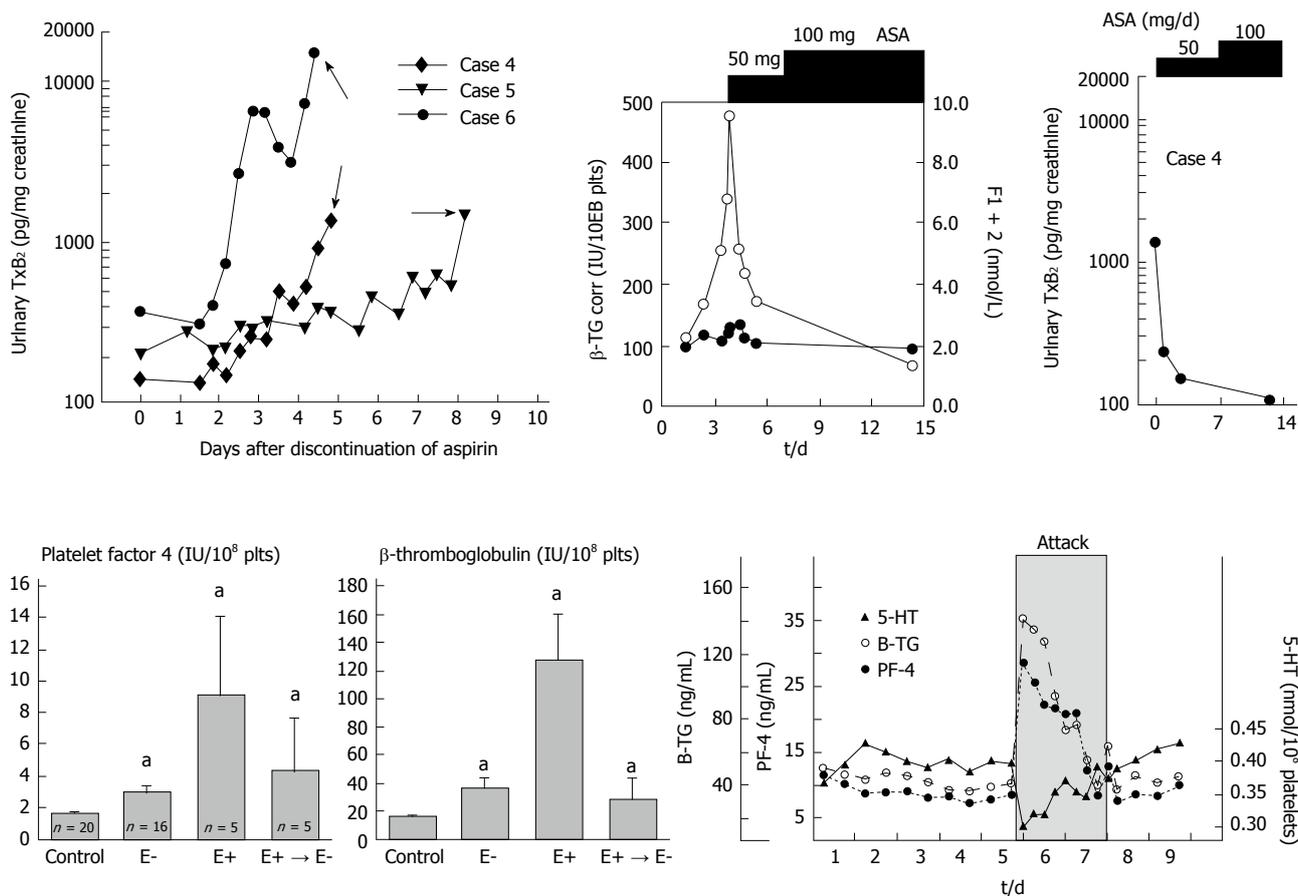


Figure 14 Vaso-active substances, prostaglandins endoperoxides and other factors released during JAK2 mutated platelet aggregation account for the inflammatory symptoms in JAK2-thrombocytopenia of ET and PV patients. Upper part: Simultaneous study of clinical signs and symptoms of erythromelalgia, platelet activation markers and increased urinary thromboxane B2 (TxB2) excretion (right) in three ET patients during attacks of erythromelalgia after discontinuation of aspirin. This was associated with large amounts of urinary thromboxane B2 (TxB2) excretion (right) and high levels of beta-thromboglobulin (middle) at time of aspirin responsive erythromelalgic symptoms in JAK2 thrombocytopenia. Erythromelalgia was successfully treated with a platelet-specific aspirin regimen of 50 mg per day, which was associated with correction of beta-TG to normal (right) and correction of TxB2 urinary excretion to normal (right). Treatment with 100 mg aspirin per day did even further decrease platelet activation markers beta-TG and TxB2 urinary excretion reaching completely normal levels^[80]. Lower part: The effects of intervention with aspirin on platelet factor 4 (PF4) and beta-thromboglobulin (beta-TG) in 20 controls, 16 cases of thrombocytopenia without erythromelalgia (E-), in 5 cases of thrombocytopenia complicated by erythromelalgia (E+) and no aspirin, and in 5 cases after curative treatment of erythromelalgia in thrombocytopenia patients (E+ → E-left and middle)^[66,67]. Decreased platelet 5-HT and increased beta-TG and PF-4 during a documented migraine attack (grey zone) demonstrating that in such patients migraine is a platelet disorder with documented *in vivo* platelet activation during the attack^[53,62,64,67]. *P < 0.05 vs control.

(Figure 15)^[75]. The platelet mediated platelet thrombophilia in JAK2-mutated thrombocytopenia of ET and PV patients was incurable and became curable by two subsequent discoveries in the history of Nature Medicine. First, Hoffmann^[75] (1897) synthesized acetyl salicylic acid (aspirin, Bayer^R), which appeared to inhibit platelet aggregation due to irreversible platelet cyclooxygenase^[1-5]. Second, aspirin cures erythromelalgia and migraine-like cerebral microvascular disturbances through platelet cyclo-oxygenase inhibition^[37,51,52] in JAK2-mutated thrombocytopenia^[76-79], could be labeled as a novel disease of platelet prostaglandin metabolism caused by JAK2 induced constitutively activated sticky platelets^[80]. Heterozygous JAK2^{V617F} mutation with low JAK2 allele burden do present with the clinical picture of ET patients at high risk for aspirin-responsive erythromelalgic microvascular circulation disturbances^[76] and low risk for major arterial and venous thrombosis^[76-80]. Low dose aspirin in ET

and aspirin/phlebotomy in PV aiming at hemotocrits of about 0.40 significantly improve the quality of life, prolongs life expectancy by the prevention of microvascular and major thrombosis, but does not influence the natural history and progression of JAK2^{V617F} trilinear MPN and CALR and MPL thrombocytopenia into myelofibrosis (Table 4)^[52,76,79]. Progression of heterozygous JAK2^{V617F} mutated ET (Step 1) into combined heterozygous and homozygous JAK2^{V617F} mutated early PV and homozygous (Step 2) mutated advanced stages of PV is due to mitotic recombination of the mutated chromosome 9p^[80]. This molecular event profoundly changed the clinical biological and pathological phenotype of trilinear MPN (Table 4 and Figure 17)^[76-80]. About one third of JAK2^{V617F} mutated MPNs with prodromal PV or with advanced masked PV is associated with significant splenomegaly, leukocytosis and major thrombosis, who are candidates for pegylated interferon as the first treatment option to

Table 4 2017 Clinical, Laboratory, Molecular and Pathobiological classification and staging of JAK2V617F trilinear Myeloproliferative Neoplasms: Therapeutic Implications

PV: CLMP stage	0	1	2	3	4	5	6
Clinical Diagnosis	Prodromal PV	Erythrocythemia	Early PV	Classical PV	Masked advanced PV	Inapparent PV: IPV → Spent phase	Post-PV MF
LAP-score, CD11B	↑	↑	↑	↑	↑/↑↑	↑	Variable
EEC	+	+	+	+	+	+	+
Red CELL MASS	N	N	↑	↑/↑↑	↑/↑↑	↑ N or ↓	Variable
Erythrocytes × 10 ¹² /L	< 5.8	> 5.8	> 5.8	> 5.8	N	N	↓
Leukocytes × 10 ⁹ /L	< 12	< 12	< or > 12	< or > 15	> 15	N or ↑	> 20
Platelets × 10 ⁹ /L	> 400	400	< or > 400	> 400	+1000	N or ↑	Variable
Bone marrow histology	EM	EM	EM	EMG	EMG	MG-MF	MF
BM cellularity (%)	50-80	50-80	60-100	80-100	80-100	60-100	↓
Grading RF	RF 0-1	RF 0-1	RF 0-1	RF 0/1	RCF2/3	RCF 2/3	RCF 3/4
Grading MF ⁵⁷	MF 0	MF 0	MF 0	MF 0	MF 1 2	MF 1 2	MF 2/3
Spleen size:							
On echogram	< 12-15	< 13	12-15	12-16	18- > 20	16 > 20	> 20 cm
Below MCL	0-3	NP	0-3	4-6	> 6	> 6	> 8 cm
JAK2 ^{V617F} load	Low ++	low ++	Moderate < 50% +	Mod/High + / ++	High > 50% ++	High → 50% ++	High → 50% ++
Granulocytes %							
Risk stratification → Therapeutic implications according to guidelines	Low	Low	Low Moderate	Inter-mediate	High early MF	JAK2 inhibitor	Post-PV MF

Source Michiels *et al*^[68,70,80] 2006, 2017. ↑: Increased; ↓: Decreased; N: Normal; +: Present or heterozygous; ++: Homozygous; RCT: Randomized clinical trial; ET: Essential thrombocythemia; PV: Polycythemia vera.

postpone or eliminate the use of hydroxyurea (Table 4 and Figure 17)^[71,76-80]. The gradual progression of JAK/Stat signalling kinase activity at the molecular level of JAK2^{V617F} mutated heterozygous into combined heterozygous and homozygous and homozygous stages of overt and advanced stages PV^[80] is associated with the acquisition of major thrombosis and constitutional symptoms in PV due to increased JAK2 mutated load, increase of activated leukocytes, and hematocrit (Table 4) on top of platelet mediated microvascular thrombotic syndrome of associated thrombocythemia^[76-80].

Vannucchi *et al*^[71] (2007) assessed the incidence of major thrombosis in a large retrospective study of 962 JAK2-MNP patients with thrombocythemia (MNP-T) subdivided in 323 PV and 639 ET patients heterozygous or homozygous for the JAK2^{V617F} mutation. 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) were excluded by definition in this retrospective analysis^[71]. 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%). 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. It should be emphasized that erythromelalgia and thrombocythemia may precede PV for several to about 10 years before latent MPN patients meet the PVSG or WHO criteria of PV^[23,37,52,68,76]. Up to date, the causal association of early functional vasomotor disturbances of erythromelalgia with

thrombocythemia and MIAs as the presenting symptom of early stage JAK2 mutated ET and PVs overlooked by internists, hematologists and neurologists^[52,76,78] and therefore not treated with aspirin until major thrombotic events of transient cerebral ischemic attacks (TIAs), major stroke, myocardial infarction had developed^[71].

The molecular pathology of JAK2^{V617F} mutated MPN can explain the gradual progression of JAK/Stat kinase activity at the molecular level of heterozygous into homozygous JAK2^{V617F} mutated MPN, which is associated with the sequential occurrence of ET, PV and MF phenotypes^[76,80,81]. This evolution of MPN disease burden in ET, PV and MF patients has significant therapeutic implications to adapt the treatment options from the use of low dose aspirin, aspirin/phlebotomy, pegylated IFN, hydroxyurea and ruxolitinib during long-term follow-up. Thromboxane A2 inhibition by dazoxiben^[82], and platelet inhibition by dipyridamol^[37,52], ticlopidin^[37,52], clopidogrel^[83] and coumarin^[37,52] are well documented to have no effect on ongoing arteriolar platelet-mediated inflammatory and thrombotic processes in JAK2-thrombocythemia of ET and PV patients^[53,60,77,80]. The association of erythromelalgia and MIAs has also been observed in congenital ET due to germline gain of function mutations in the TPO, JAK2 and MPL gene^[79,80,81]. These germline gain of function mutation constitutively activate bone marrow megakaryopoiesis *via* the MPL receptor (TPO-receptor) signalling mechanism and increased production of hypersensitive sticky platelets as the cause of autosomal dominant aspirin responsive sticky platelet syndrome^[70,81]. At platelet counts from below to above 1000 × 10⁹/L erythromelalgic thrombotic thrombocythemia (ETT) is complicated by

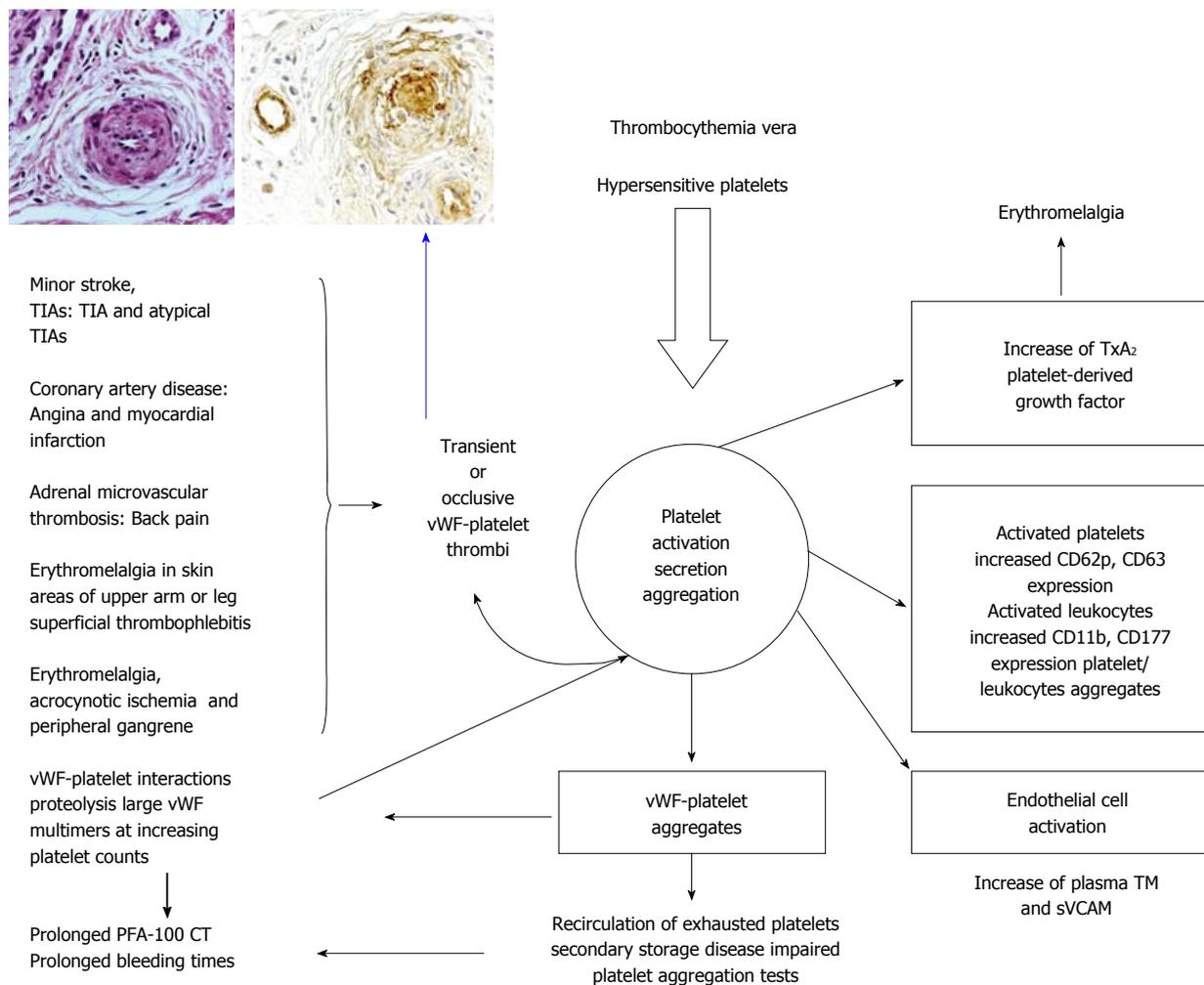


Figure 15 Shear induced platelet activation of constitutively JAK2^{V617F} hypersensitive sticky platelet with increased CD62p and CD 63 expression) in thrombocythemia vera of JAK2^{V617F}-positive ET and PV patients is the cause of a broad spectrum of platelet-mediated arteriolar inflammatory and thrombotic manifestations of erythromelalgia, digital ischemic complications, superficial thrombophlebitis, MIAs, TIAs, adrenal microvascular thrombosis and TIAs or even stroke and acute coronary syndrome in particular when thrombocythemia^[79,80] is associated with increased activated leukocytes and erythrocyte count of polycythemia vera (increased cellular blood viscosity (Figure 12). In this process of *in vivo* platelet activation and secretion, reversible VWF-platelet aggregates activate endothelial cells to secrete thrombomodulin (TM) and sVCAM^[79,80], whereas secreted PDGM accounts for the fibromuscular intimal proliferation (inset left) followed by occlusion of arterioles by VWF-rich platelet thrombi (inset right). After reversible aggregation the platelets recirculate as exhausted platelets with secondary storage pool deficiency and impaired platelet functional defects. The platelet - Von Willebrand factor (VWF) interactions leads to proteolysis of large vWF multimers at increasing platelet counts from below to above $1000 \times 10^9/L$ (Figure 13 right and Figure 16 peak 1 and 4).

spontaneous hemorrhagic thrombocythemia (HT) or paradoxical ETT/HT due to acquired von Willebrand disease type 2A (Figure 15)^[53,77,78], which is reversible by reduction of platelet counts from above to below or $1000 \times 10^9/L$ or to normal preferentially with interferon (Pegasys[®]) or anagrelide in JAK2, MPL and CALR thrombocythemias^[37,53,61,69,79,80]. CALR and MPL mutated thrombocythemias usually present with high platelet count around or above $1000 \times 10^9/L$ complicated by ETT/HT or HT. Since CALR and MPL thrombocythemias have no features of PV the incidence of major arterial and venous thrombosis is low^[80,81].

specific aspirin maintenance regimen of 50 to 100 mg OD, which is associated with correction of TxB2 urinary excretion to normal leaving the prostacycline synthesis in endothelial cells intact (Figure 14, Van Genderen *et al*^[62-64]). Inhibition of platelet ADP (P2Y12) receptor by clopidogrel^[83] leaving cyclooxygenase activity intact (Figure 13) does not prevent shear induced spontaneous activation of JAK2 induced hypersensitive sticky platelets in the endarterial circulation in ET and PV patients in the absence of vascular pathology or arterioclorotic disease.

ROLE OF DUAL ANTIPLATELET THERAPY IN ACUTE CORONARY SYNDROMES

Erythromelalgia is successfully cured by a platelet-

ASPIRIN AND ADP (P2Y12) RECEPTOR INHIBITORS IN ACUTE CORONARY SYNDROME

Low dose aspirin 75 mg OD vs placebo in 796 patients

1978

Three year history of burning painful red or blue toes and forefoot sole of the right foot (erythromelalgia)

Spontaneous hemorrhages of large ecchymoses on the chest and subcutaneous hematomas

Erythromelalgia, which characteristically disappeared by treatment with low dose aspirin

During aspirin treatment the platelet counts increased from $1.101 \times 10^9/L$ to $1.700 \times 10^9/L$

After cytoreduction of platelet counts to normal by busulphan the symptoma did not recur

May 1992. Third relapse of thrombocythemia

Platelets $861 \times 10^9/L$. Aspirin responsive erythromelalgia big toe/forefoot

September 1992

Severe ischemic attacks of unsteadiness

Monoparesis of the right arm and dysarthriaparadoxically occurred

Subsequent treatment with aspirin was associated with:

No recurrence of cerebral ischemic attacks

Increase of platelet count to about $1500 \times 10^9/L$

Persistence of bleeding symptoms

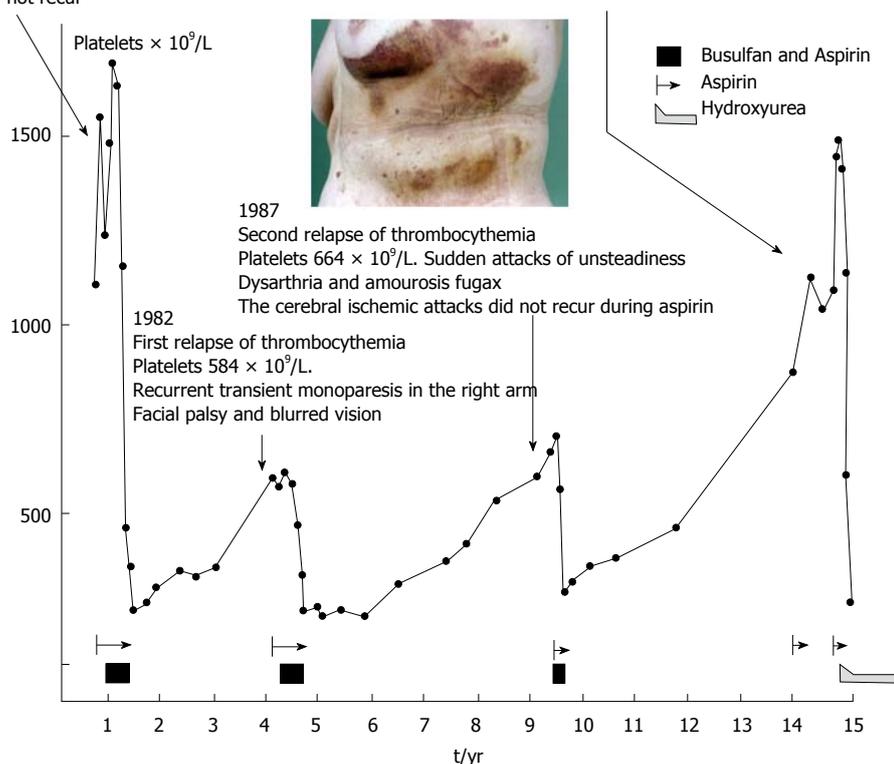


Figure 16 Longterm observations in a 72-year-old woman with a three years history of burning painful red or blue toes and forefoot sole of the right foot (erythromelalgia) presented in December 1978 with spontaneous hemorrhages of large ecchymoses on the chest and subcutaneous hematomas. Paradoxical 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. The first episode of erythromelalgia for 3 years followed by simultaneous occurrence of thrombotic thrombocythemia and hemorrhagic thrombocythemia at platelet counts around $1100 \times 10^9/L$ in 1982. Aspirin was effective at peak 1 for the relief of erythromelalgia, which was associated with a further increase of platelet count to about $1500 \times 10^9/L$. During periods of thrombocythemia at peak 1, 2, 3 and 4 busulphan induced complete remissions of thrombocythemia resulted in normal platelet counts below $400 \times 10^9/L$, which was associated with no recurrence of erythromelalgia when not on aspirin. At peak 2 and 3 in the figure, recurrence of a second and third episode of thrombotic thrombocythemia occurred at platelet counts between 600 and $800 \times 10^9/L$. In 1992 at peak 4 the patient suffered from an episode of thrombotic and hemorrhagic thrombocythemia at platelet counts of $1040 \times 10^9/L$. Again aspirin relieved the erythromelalgia, which was associated with a further increase of platelet count to to around $1500 \times 10^9/L$ but the hemorrhagic manifestation persisted which associated with acquired von Willebrand factor deficiency type 2A (acquired von Willebrand syndrome: AVWS and disappeared after correction of platelet count to normal) (Figure 15).

with unstable angina or non-Q myocardial infarction in the presence of arterioclerotic vascular pathology was effective to reduce the probability of death or myocardial infarction during one year follow-up (Figure 18)^[84]. After revascularization or stent implantation in the percutaneous cutaneous intervention (PCI) setting the endothelial cell layer of the treated coronary artery has been removed with the consequence of platelet adhesion and aggregation as the cause of reocclusion if left untreated. We hypothesize that treatment with ADP (P2Y12) receptor blocker allow platelets to adhere to the subendothelium by VWF-GPIb and collagen receptor mediated adhesion to the subendothelium, while ADP (P2Y12) receptor inhibition does inhibit the

propagation of platelet aggregation thereby preventing reocclusion of the damaged coronary artery after PCI (Figure 13). Treatment of ACS patients with successful stent implantation (PCI) in 517 patients randomized for Marcoumar (INR 3.5-4.5) + aspirin 100 mg BID vs Ticlopedine 250 mg BID + aspirin 100 mg BID showed superiority of dual antiplatelet therapy of platelet cyclooxygenase and ADP (P2Y12) receptor inhibition as compared to aspirin and vitamin K antagonist (Figure 18)^[85]. The conclusion is that oral anticoagulation does not play a role in the reocclusion of coronary artery after PCI as compared to dual antiplatelet therapy and ticlopedin is superior to aspirin in the PCI setting of stent implantation. This could be confirmed in the

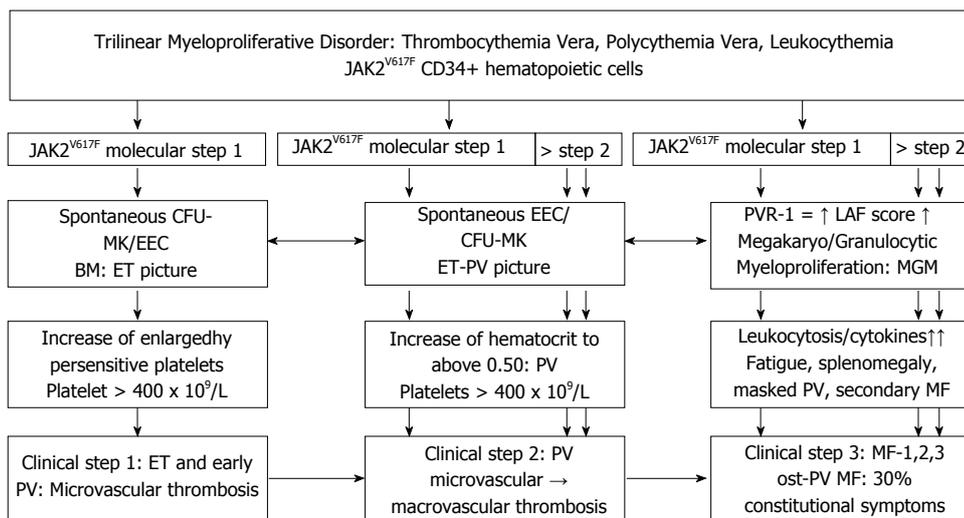


Figure 17 The molecular etiology of JAK2^{V617F} heterozygous mutated essential thrombocythemia (essential thrombocythemia picture Step 1) and evolution into combined heterozygous/homozygous or homozygous JAK2^{V617F} mutated sequential stages of prodomal polycythemia vera classical PV (ET/PV pictures) and post-polycythemia vera myeloid metaplasia of bone marrow and spleen complicated by chronic idiopathic or secondary myelofibrosis.

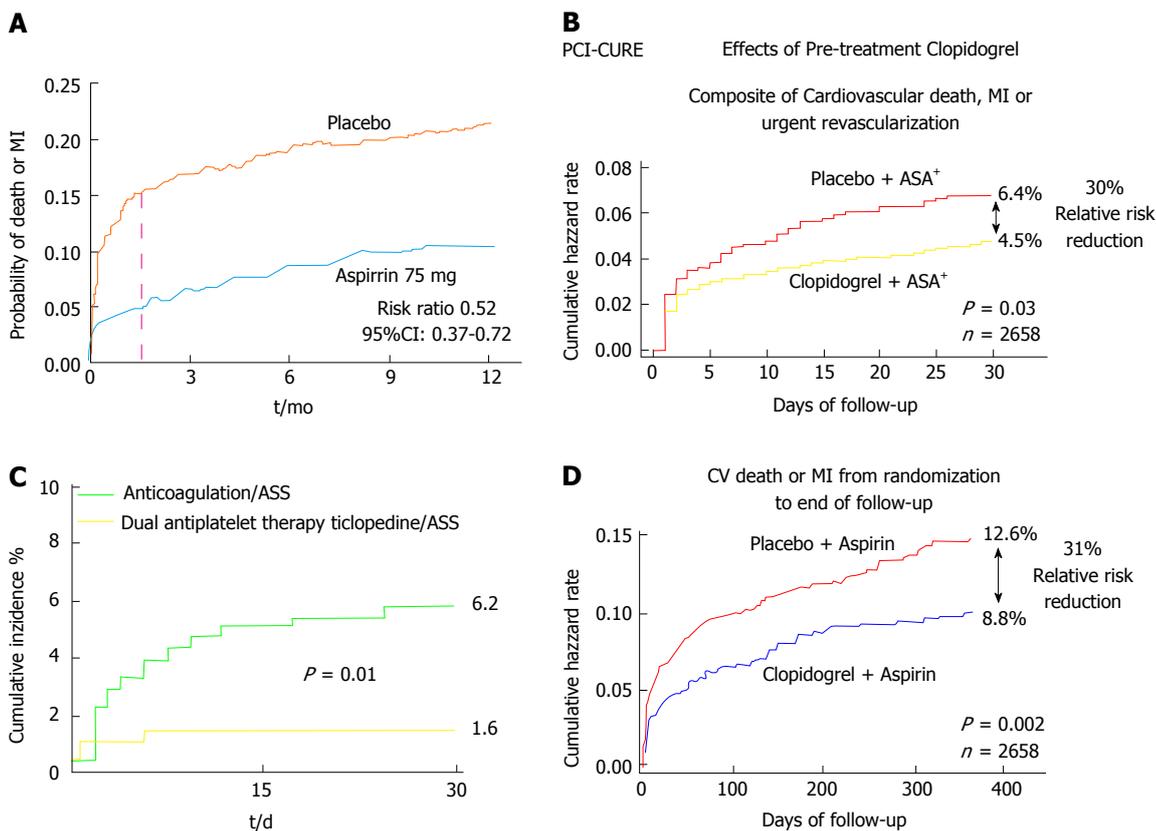


Figure 18 Low dose aspirin 75 mg OD vs placebo in 796 patients with unstable angina or non-Q myocardial infarction in the presence of arterioclerotic vascular pathology was effective to reduce the probability of death or myocardial infarction during one year follow-up. A: Aspirin 75 mg OD vs placebo in 796 patients with unstable angina or non-Q myocardial infarction (MI) reduced the probability of death or MI from about 20% to 10% during 1-year follow-up^[82]; B: Aspirin/marcoumar vs aspirin/ticlopedin after percutaneous cutaneous intervention (PCI) reduced the combined events of cardiac death, MI, bypass or recurrent PCI from 6.2 to 1.6% after 1-mo follow-up^[83]; C: Aspirin/placebo vs aspirin/clopidogrel in 2625 treated PCI patients reduced the composite of cardiovascular death, MI, or urgent revascularization from 6.5% to 4.5% in the PCI-CURE study^[84]; D: The extended substudy of the PCI-CURE reduced the combined cardiovascular death and MI reduced from 12.6% to 8.8% after 1-year follow-up^[84].

Clopidogrel in Unstable Angina to Prevent recurrent Events Trial (PCI-CURE) in non-ST-elevation acute coronary syndrome patients. The PCI-CURE study examined whether the addition of clopidogrel to

aspirin (dual aspirin-clopidogrel) vs aspirin alone in the PCI setting improves the outcome in terms of cardiovascular death, myocardial infarction (MI) or urgent revascularization (Figure 19)^[86]. Clopidogrel was

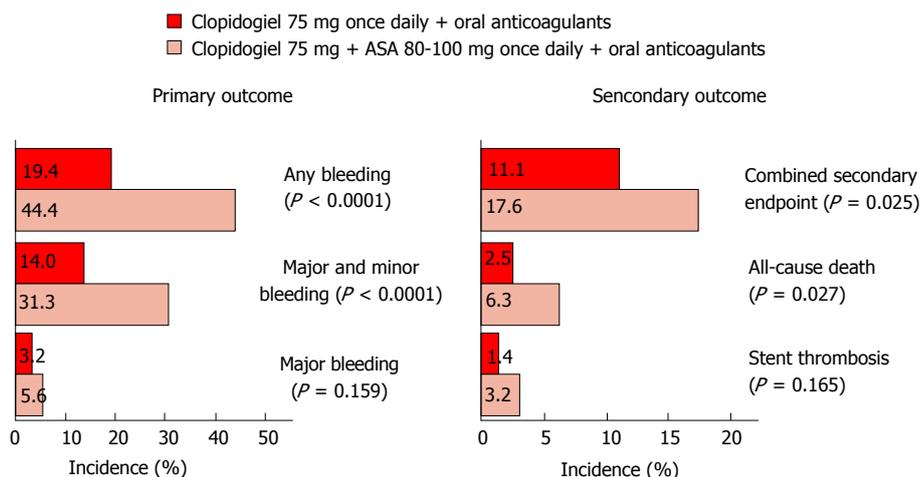


Figure 19 Dual clopidogrel and coumarin compared to triple clopidogrel coumarin and aspirin in 573 patients undergoing PCI receiving oral anticoagulation for another reason in the WOEST study was equal in terms of death, reocclusion and revascularization but superior for dual clopidogrel/coumarin in terms of any bleeding (44.4% vs 19.4%), major and minor bleeding (31.3% vs 14%) and major bleeding (5.6% vs 3.2%)^[85].

given orally in an immediate dose of 300 mg followed by a maintenance dose of 75 mg once daily. There was a 30% relative risk reduction (RRR) from 6.5% to 4.5% in 2658 treated PCI patients during the first month follow-up and a 31% RRR from 12.6% to 8.8% after one year follow-up in a subgroup of PCI Cure patients (Figure 18)^[86]. The likely explanation is that ADP inhibition by clopidogrel on top of aspirin does allow platelet adhesion to subendothelium of the coronary artery after PCI, but prevent subsequent platelet mediated reocclusion of the coronary artery after PCI by the double aspirin and clopidogrel inactivated platelets. As shown in Figure 13 the ADP induced pathway of platelet inactivation is not needed in aspirin responsive erythromelalgia and microvascular ischemic disturbances in JAK2-mutated thrombocythemia. In the PCI setting, the ADP (P2Y12) pathway inhibition of platelet by clopidogrel is of critical importance in the reduction of the reocclusion rate after PCI, while the aspirin sensitive AA pathway plays a minor role (Figure 13). The critical question is whether aspirin is needed on top of clopidogrel in the PCI setting to prevent reocclusion. The WOEST evaluated the safety and efficacy of dual clopidogrel/oral anticoagulation (Clop/OAT) therapy ($n = 284$) for one year after PCI compared with triple clopidogrel/aspirin/coumarin (Clop/Asp/OAT) in patients undergoing PCI receiving oral anticoagulation for another reason atrial fibrillation in particular ($n = 289$)^[87]. The primary efficacy endpoint was a combined end point of minor, moderate or major bleeding complication during the initial hospitalization and one year follow-up. The secondary efficacy endpoint was a combined event of death, myocardial infarction, stroke, systemic embolization and target vessel revascularization during one year follow-up (Figure 19). After one year follow-up, any bleeding had occurred in 54 patients (19.4%) in the dual Clop/OAT patients as compared to 126 (44.4%) in the triple Clop/

Asp/OAT patients^[87]. The incidence of major and minor bleeds was significantly higher in the triple Clop/Asp/OAT group; 31.2% as compared to the dual Clop/OAT group, 14.0%^[87]. Secondary outcome events occurred in 31 patients (11.1%) in the double Clop/OAT group and in 50 patients (17.6%) in the triple Clop/Asp/OAT group. At one year, 7 patients (2.5%) in the double Clop/OAT and 18 patients (6.3%) in the triple Clop/Asp/OAT had died from any cause (Figure 19)^[87]. In patients taking oral anticoagulation and undergoing PCI, dual Clop/OAT is superior to triple Clop/Asp/OAT treatment in terms of bleeding complications and there was no evidence of increased thrombotic risk after PCI without the use of aspirin.

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