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Polycythemia Vera era 1939-1998 from Berkeley to Rotterdam and Change of PVSG into ECP Criteria of Myeloproliferative Disorders ET, PV and PMGM: Proceedings of the First Rotterdam MPD Workshop 1998 and Beyond

Jan Jacques Michiels*

Goodheart Institute & Foundation in Nature Medicine & Health, Rotterdam, Netherlands, Europe

Abstract

The PVSG defined in 1975 the myeloproliferative disorders (MPD) essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF) or agnogenic myeloid metaplasia (AMM) are three distinct disease entities with regard to clinical manifestations, natural history and outcome in terms of life expectancy. The European Clinical and Pathological (ECP) criteria demonstrated that fibroblast proliferation in PVSG defined PMF and post-ET and post-PV myelofibrosis is polyclonal indicating that myelofibrosis (MF) is secondary in all variants of myeloproliferative syndromes. By including bone marrow histopathology the ECP defined between 1998 and 2000 the prefibrotic MPDs ET, PV and primary megakaryocytic granulocytic myeloproliferation (PMGM) are three distinct myeloproliferative disease (MPD) entities. Clinical, hematological and morphological characteristics, in particular megakaryocytopoiesis and bone marrow cellularity, reveal diagnostic features, which enable a clear-cut distinction between two prefibrotic MPDs ET and PV versus PMGM as the third distinct MPD entity. Life expectancy is normal in normocellular ET, normal during the first ten years and compromised during the second ten years follow-up in PV, but shortened in the prefibrotic hypercellular stage of PMGM. First line treatment options anno 2000 were control of platelet function with low-dose aspirin in ET, low-dose aspirin on top of phlebotomy in PV and a wait and see strategy in the prefibrotic and fibrotic stages of PMGM. Pegylated Interferon (IFN) was proposed in between 1998 and 2000 as the first line non-leukemogenic treatment option in prodromal PV and early or classical stages of PV. Because of its leukemogenicity hydroxyurea should be postponed as long as possible and to be used in progressed MPD restricted to the hypercelluar stages of PV and ET for the treatment of uncontrolled thrombocytosis, leukocytosis splenomegaly and constitutional symptoms. These conclusions could be confirmed in the Dutch updates since 2000, are still valid and did not change significantly between 2008 and 2018 due the discovery of JAK2, CALR and MPL driver causes of MPN between 2005 and 2013.

Keywords: Megakaryopoiesis; Erythropoiesis; Granulopoiesis; Myeloproliferative disorders (MPD) essential thrombocythemia; Polycythemia vera; Agnogenic myeloid (megakaryocytic/granulocytic) metaplasia (AMM)

Introduction

The first Rotterdam Workshop on Myeloproliferative Disorders (MPD) 1998 was organized by Jan Jacques Michiels and Juergen Thiele and started with the first International Scientific State of the Art Session of the International PVSG chaired by Jan Jacques Michiels. Dr. Michiels highly welcomed Dr. Nathaniel Berlin as invited honorary guest to represent the PVSG and to introduce the PVSG concept of polycythemia vera (PV) for the members of the European Working Group on MPD (EWG.MPD) of the European Hematology Association (EHA).

Dr. Nathaniel Berlin joined and opened the "First Scientific Meeting of the International PVSG" held on March 4, 1998, Thursday evening from 1800 to 2000 hours at Novotel, Rotterdam. The program was organized by Dr. Michiels reflecting the current activities of the International PVSG:

1. Berlin NI: The Continuation of the International PVSG, links between the USA and Europe.
2. Fruchtmann S: Rationale for the use of Hydroxyurea and supplemental phlebotomies in PV.
3. Silver RT: First-line treatment for PV with interferon-alpha.
4. Thiele J and Kvasnicka HM: Bone marrow histopathology as a specific clue to ET, PV and PMGM.
5. Michiels JJ: Van Genderen P.J.J. and Van Vliet H. Erythromelalgic thrombotic thrombocythemia: ETT.
6. Michiels JJ: Changing insights in the natural history, diagnosis and treatment options in PV ET and PMGM.
7. Polycythemia Vera 1939-1998: From Berkeley to Rotterdam (Berlin NI).

Firstly, I want to thank Dr. Michiels for inviting me to this meeting and for asking me to open the First Business meeting of the International PVSG 1998. I am delighted to be here to see friends of long standing. Richard Silver, who left the National Cancer Institute just before I got there and Yves Najean who in the middle 1960s came to the NIH with the late Catherine Dresch, to discuss erythrokinetics and Steven Fruchtmann who maintains the PVSG data. And last it is great pleasure to meet and get know those of you who have been until today names

***Corresponding author:** Jan Jacques Michiels, Goodheart Institute & Foundation in Nature Medicine & Health, Rotterdam, Netherlands; Tel: +31-626970534; E-mail: goodheartcenter@upcmail.nl

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on papers. I am particular pleased to see the leaders of the French and Italian Polycythemia Vera study Groups here, I would not dare to try to pronounce the groups names in French or Italian that courtesy if nothing else requires. Turning now to the two themes I wish to present today (March 13, 1998), the first is a brief history more personal, the second a glimpse into what I believe this group can do or perhaps what I would like to see it do. My association with the polycythemias began in June 1947 at the Donner Laboratory of the University of California as a graduate student of John Lawrence and at the same time began my lifelong friendship with Louis Wasserman who was working at the laboratory that summer (Figure 1). The modern era in the understanding and treatment of PV began in 1939 when Lawrence treated the first patient with P^{32} [1]. The results in the first two patients were reported in 1940. Each had an excellent response; in fact one of these patients was alive when we published our review in 1953 in Medicine [2]. Today one can say it was not necessary to demonstrate the effectiveness of P^{32} in more patients. These two patients are in themselves sufficient. It was at the Donner Laboratory that Wasserman began the measurement of arterial oxygen saturation as a diagnostic tool where Huff initiated the studies on kinetics of erythropoiesis with radioactive iron and I began the use of blood volume measurements with P^{32} labeled red cells in diagnosis and management [3-5]. It was from the patients treated at the Donner Laboratory that we were able to report in 1953 for the first time the life expectancy in P^{32} treated patients (2) and probable had the data to show that it approached normal, although it was not until 1991 that Rozman showed that this was so in Spanish patients [6]. This has been confirmed by Najean in French patients and by the Italian group in Italian patients [7,8].

Wasserman favoured the concept that PV is a neoplastic process of the whole bone marrow and proposed a hypothetical concept of the course of PV (Table 1 and Figure 2) [9].

Wasserman distinguished at least five subsequent stages in the natural history of PV.

Stage 1

Pure erythrocythemia due to myeloproliferative disease with increased haemoglobin, hematocrit and red cell mass, normal leukocyte and platelet counts, and no splenomegaly on palpation.

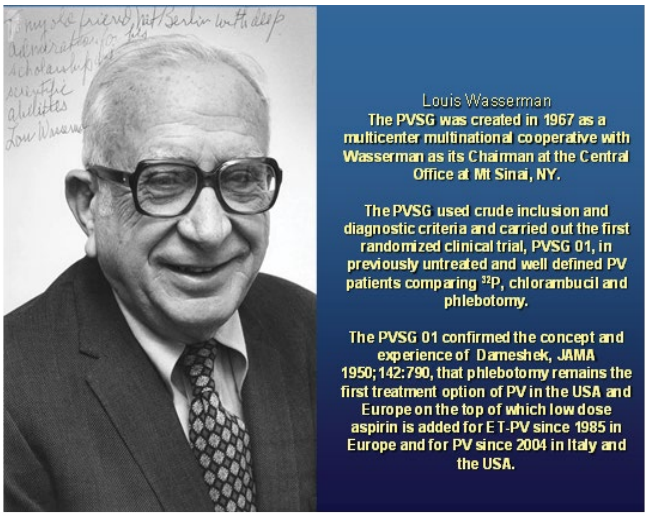

Figure 1: Louis Wasserman, Founder of the PVSG. Photo courtesy of Nathanial I. Berlin at the occasion of his active participation at the First Rotterdam Workshop on Myeloproliferative Disorders (MPD) of the European Working Group on Myeloproliferative Disorders: EWG.MPD in Rotterdam, March 1998.

Comparable incidence of Acute Leukemia by Protocol and Treatment in the Phlebotomy arm of PVSG 01 and HU PVSG 08 according to Fruchtmann				
Parameters	**Protocol**	**Patient**	**Total Events**	**%**
On study Events first 795 weeks of study	HU-NPT 08	51	3	5.9
	Phleb 01	134	2	1.5
All events	HU-NPT 08	51	5	9.8
	Phleb 01	134	5	3.7

Table 1: Comparable incidence of Acute Leukemia by Protocol and Treatment in the Phlebotomy arm of PVSG 01 and HU PVSG 08 according to Fruchtmann.

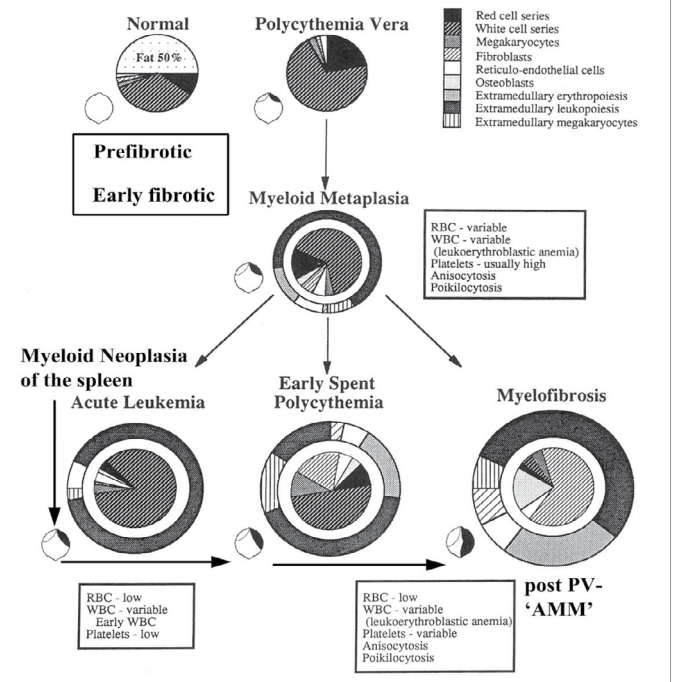

Figure 2: The concept of Wasserman on the course of polycythemia vera of the fibroblasts1995[9]. In PV, the hyperplastic marrow is a neoplastic panmyelosis that expands to encompass the total marrow space at the expense of the fat cells. Blood examination reveals an erythrocytosis and usually a granulocytic leukocytosis, thrombocytosis, and immature red and white cells. With continued hyperactivity, the dominant extra-osseus potential marrow becomes functionally active and the site of extramedullary hematopoiesis (stage of myeloid metaplasia) as the spleen and liver enlarge. The bone marrow shows diminuation in erythropoiesis and secondary proliferation. With time progressive changes in cellular composition of the marrow occur in early spent phase PV. Fibroblastic or osteoblastic proliferation may encroach on hematopoietic cell activity with reduction of erythropoiesis. Progressive dysmature megakaryoytic fibroblastic proliferation and progressive splenomegaly is the hallmark of advanced myeloid metaplasia associated with anemia and a leukoerythroblastic blood picture. Spent phase PV, pronounced splenomegaly, myelofrosis and acute leukemia are all depicted as stemming from a common stage of myeloid metaplasia in the bone marrow and extramedullary metaplasia in the spleen and liver.

Stage 2

The polycythemic stage (prefibrotic)

Stage 3

Progressive myeloid metaplasia with different grades of reticulin and collagen fibrosis in the bone marrow, and progressive splenmegaly in about one third of the cases during long-term follow-up. The polycythemic stage and subsequent transformation into progressive myelofibrotic myeloid transformation may elapse from 5 to 25 years.

Stage 4

Spent phase PV reflects a period of normal red cell values (so-called spontaneous remission of PV) with large spleen enlarged liver, pronounced thrombocythosis is frequent with bizarre giant platelets and granulocytic leukocytosis.

Stage 5

Post-PV myeloid metaplasia, myelofibrosis (dry tap on aspiration) with various degrees of leukoerythroblastosis, anemia and pronounced splenomegaly (Figure 2) [9].

The PVSG was created in 1967 as a multi-institutional multi-national cooperative with Wasserman as its Chairman and the Central Office at Mt Sinai. I served for a time as the vice-chairman until Paul Berk joined the staff of Mt Sinai. The PVSG developed the diagnostic criteria that are widely used and cited [10]. There are more than 280 citations in the literature, certainly some are self-citations, we developed an algorithm for the evaluation of an elevated hematocrit [10,11], initially we called it a decision tree and we developed and carried out what has become known as PVSG 01-protocol – our first randomized study comparing P^{32}, chlorambucil and phlebotomy and we developed recommendations for treatment [12]. We have published reports from the PVSG in 1976, 1987 and 1997 in Seminars in Hematology and in 1995 published our book Polycythemia Vera and the Myeloproliferative Disorders edited by Wasserman, Berk and Berlin [9,13-16]. We did not do a systematic review of symptom relief, nor changes in spleen or liver size or blood pressure. That was done at Berkeley in much smaller group of P^{32} treated patients [9]. We did both on study and off study analyses of events in the NEJM paper on chlorambucil [12] but subsequently all our efforts of the PVSG 01 protocol are for survival on study but did comment that all event analyses were done and did not change the conclusions. We did comment on differences in the rate of withdrawal alive from study was the highest in the phlebotomy treated patients, which is mentioned in the NEJM paper on chlorambucil [12].

Today we know that life expectancy in hydroxyurea treated and PVSG defined PV patients' approaches normal but questions remain. The two recent papers in Blood by Najean and Rain are important [7,17]. They raise major questions about how best to treat initially. I will not attempt to discuss treatment options of the later phases except that we did a study of the treatment of the acute leukemia phase-it was not successful and we discussed extensively and in fact wrote a protocol for the treatment of the splenomegalic myeloproliferative phase that proved to be difficult to carry out. The Najean paper in the November 1, 1997 issue of Blood provides additional data to answer the question is hydroxyurea a leukemogen, which I think it is [17].

Today anno 1998 the questions, which I pose are:

1. Shall we revise the PVSG diagnostic criteria as Dr. Pearson [18] and our host Dr. Michiels have suggested [19].
2. How shall blood volume measurements be reported, as ml/kg or as some function of body composition.
3. How shall they be interpreted, namely when is the line crossed that they are diagnostic.
4. When are they necessary (desirable) either initially or during the course of management.
5. Can we as a group state that hydroxyurea is leukomogenic.

In the process of developing treatment guidelines what criteria should be used in the decision making process, what is best for the patient in terms of life expectancy and what produces the greatest symptomatic relief, what is the simplest for the treating physician and does not compromise the outcome, which requires the least compliance by the patient as has been suggested by Najean and others but Dick Silver will make the case for interferon [20].

Last we must all acknowledge that the molecular biology era is here. I do not know where it will lead. It has already given us the finding that PV is a clonal disorder which Lawrence and I discussed in the early 1950's in terms, is it a neoplasm. In closing I wish to pay tribute to the French and Italian Groups and their leaders Najean and Barbui. They have moved the leadership in the study of PV from the USA (Spivak & Silver) to Western Europe. I look forward to the ECLAP study to answer the question can aspirin lower the hemorrhagic and thrombotic complications [21]. As I read and re-read some of the recent literature [22-41], I must say that I do not come before you today with original ideas of what should be done. I can only add my choice to that of others and last for the sin of omission in failing to recognize many of the contributions of those here today I apologize and ask forgiveness and for not mentioning only the senior authors in this talk I apologize.

Controversies in the Management of MPDs: What is the Progress since the Demise of the PVSG in 1997 (Fruchtman SM)

As far as I can derive from the issue in Seminars of Hematology in January 1997 [42], there is no residual activity of the PVSG and all PVSG protocols are closed many years ago [11,43]. We have discussed and learned much from the results of the PVSG studies at the various meetings of the EWG.MPD since 1994 summarized in The Chronic Myeloproliferative Disorders CMPD): Essential Thrombocythemia, Polycythemia Vera and Megakaryocytic Myeloid Metaplasia. Leukemia & Lymphoma volume 1996 Guest Edited by Jan Jacques Michiels [19]. From these publications, we got the very strong feeling to refrain from all old PVSG studies including the old studies of Najean from Paris, which all are extensively reviewed in the Leukemia & Lymphoma issue of 1996 [35]. The main conclusion from the PVSG is that Hydroxyurea (HU) is currently recommended as the least leukemogenic approach and supplement to aspirin/phlebotomy for newly diagnosed patients, who are at risk for thrombosis and for PV patients with progressed myeloproliferative disease at presentation or during follow-up. The landmark studies of Najean in Blood stresses the toxicity of long-term HU treatment in PV [44,45]. In view of the available data of Silver on interferon in PV, we now have to address the question how to design a worldwide acceptable study protocol in PV [46]. How should such a study design in PV look like to address the key question not to undertreat or over treat PV patients during the very long-term follow-up of 10, 20, and even more than 30 years. How to organize and to fund such long-term PV-studies, the very large retrospective Italian study of more than 1200 PV- patients in the Annals of Internal Medicine heavily treated and followed up between 1975 and 1995 does not provide us the relevant information in offering tools for the best treatment modality in PV [8]. Michiels reviewed The Myeloproliferative Disorders ET, PV and megakaryocytic myeloid metaplasia in 1996 [23]. Michiels, Barbui and Finazzi described the vascular complications in patients with PV in Seminars in Thrombosis 1997 from the standpoint of the least toxic approach by experienced and sensitive doctors from the literature [47,48]. Since the first annual meeting of the European Hematology Association (EHA) in 1994, Michiels founded and organized the annual Scientific Working Group (SWG) on Myeloproliferative Disorders

(MPD) at annual EHA Meetings. Within the EHA SWG.MPD Patrono, Landolfi and Michiels designed ECLAP (European Collaboration on Low-dose Aspirin in Polycythemia) study between 1994 and 1996 [21]. Landolfi and Patrono reviewed the use of aspirin in PV and ET: current facts and perspective as the extension of the Dutch Collaboration on Low Dose Aspirin in ET (Dutch-CLAT study) [32]. Marchioli et al assessed the feasibility of randomized clinical trials in PV [37]. Landolfi and Marchioli wrote the ECLAP protocol (Figure 3) [21]. The ECLAP study protocol was approved by the European got funding from the European Community within the BIOMED 2P program (Contract No. ERBBMH4CT961433) and started in 1997.

The major goals of the PVSG was to search for the least toxic approach and to establish criteria for the diagnosis of MPDs enter patients onto clinical trials and determine the optimal treatment strategy with the agents commonly used in that era [49,50]. Since the demise of the PVSG due to the loss of no funding, leadership in design and performance of pivotal clinical trials in MPDs has shifted to Europe, under the leadership of the innovative clinical investigators Pearson, Georgii, Thiele, Barbui, Landolfi and Michiels. Clearly, the PVSG defined the ground work for establishing clinical criteria for the ET, PV and PMF diseases to be studied and that multi-institutional, international trials were required to accrue sufficient numbers of patients to be entered into trials and work collaboratively and established that pharmaceutical agents patients were randomized to influence long term prognosis and transformation to acute leukemia. The use of clinical criteria to establish

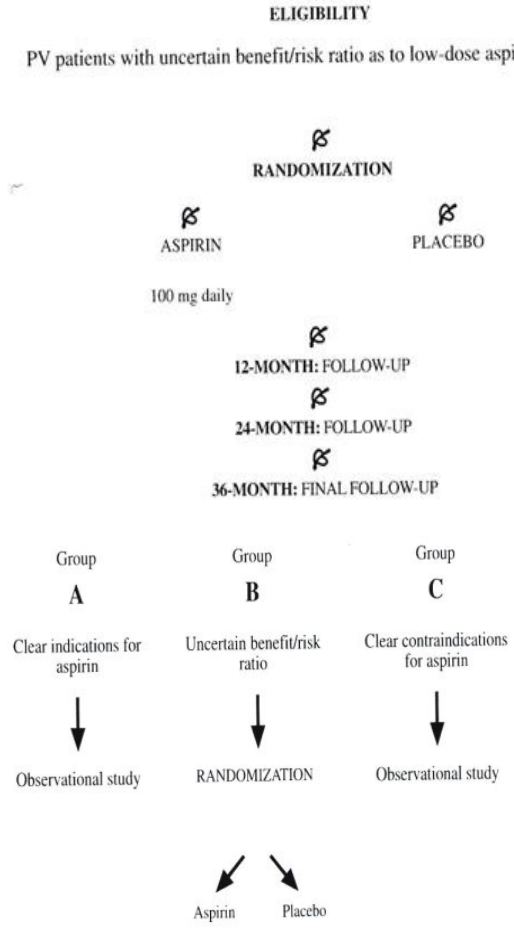

Figure 3: ECLAP Protocol.

diagnosis was extremely important in establishing similar cohorts of patients to be entered onto trials, but it lead to confusion amongst the practicing community that concluded that unless the criteria were met, a patient could not carry the diagnosis. It required an educational effort to instruct physicians that this was a misconception.

The notion of multi-institutional, international, prospective trials was unique at that time, but clearly the PVSG established the frame work and communicative infrastructure to create a creative and successful working environment. The PVSG O1 trial set to rest the notion that all patients needed cytoreductive therapy, due to the observation of increased acute leukemia occurring in patients so treated. Unfortunately patients treated with phlebotomy alone had a higher rate of thrombotic complications, with many of these thrombosis causing death. Thus, the PVSG established that thrombosis and acute leukemia were the major causes of morbidity and mortality in Ph-MPDs. In order to establish a cytoreductive regimen that was safe for long term use, the PVSG studied hydroxyurea to address the controversial question whether or not hydroxyurea is leukemogenic.

The PVSG data and other investigators report that the incidence of leukemic transformation in hydroxyurea treated patients in the PVSG 08 (Table 2) [42,43] is higher than presented in the phlebotomy arm of the PVSG 01 study. Such studies must report other therapies the patient received, due to the observation by both Najean [44,45] and Murphy [50] that an extremely leukemogenic formula is when one cytoreductive therapy especially alkylators is followed by hydroxyurea. More recently, anagrelide has been investigated in a series of abstracts presented at the American Society of Hematology (ASH 1997). We have reported on its efficacy in preventing both thrombosis and hemorrhage in these patients, and recently Tefferi et al published on its long term safety of anagrelide in ET regarding safety, efficacy and absence of leukemogenicity [46].

Erythromelalgic Thrombotic Thrombocythemia in ET and PV (Michiels JJ, Van Vliet HHDM and Van Genderen PJJ)

Michiels discovered between 1975 and 1985 that aspirin responsive erythromelalgia and its ischemic complications and aspirin responsive migraine-like atypical transient ischemic cerebral ocular (MIAs) as well as coronary ischemic attacks are specific clinical manifestations of thrombocythemia vera caused by platelet-mediated arteriolar inflammation and thrombosis in ET at platelet count above 400 × 10/L and in PV patients associated with thrombocythemia at platelet count above 350×10^9/L [27,48,49]. Michiels and Ten Kate recognized

1	Polycythemia vera or erythremia is characterized by initial erythrocytosis (red cells >6 × 10^9/l), which is usually accompanied by leukocytosis (>12 × 10^9/l) thrombocytosis (>400 × 10^9/l), splenomegaly, plethora and ruddy cyanosis.
2	The average age in 272 cases of PV was 48 years with a male to female ratio of 1.8:1
3	The interval of clinical features and diagnosis of PV may vary from a few days to 30 years. PV patients may have splenomegaly for 20 to 30 years before symptoms caused them to be sending for treatment.
4	The PVSG and WHO use increased red cell mass as a major criterion for PV (mean age 60 years) and therefore overlook the early stages of PV when the
5	ET, erythromelalgia, may preceed PV for a few to more than 10 years.
6	Presentig features of microvascular disturbances have been overlooked as a pathognominic clue to early stage PV until major thrombosis occur.

Table 2: Definition of Polycythemia Vera from Wasserman 1954 to PVSG 1975 and Michiels 1980 RCP and 2000 ECP criteria for PV and ET.

that erythromelalgic thrombotic thrombocythemia (ETT) in ET and PV patients (Figures 4 and 5) had overlapping bone histology features (Figure 6). Bone marrow histology in ET is featured by normcellular increase of large mature megakaryocytes (M) or shows a hypercellular myeloproliferation of increased erythropoiesis and clustered mature megakaryocytes (prodromal PV). Bone marrow histology in PV is featured by increased eyrthropoisis and clustered mature large megakaryocyte proliferation (EM) in early stage classical PV but increase of trilinear erythro-megakary-granulocytic (EMG) myeloproliferation in hypercellular PV. The morphology and clustering of large mature and pleiomorphic megakaryocytes are indentical in ET, prodromal PV and early stage PV in both ET and PV who presented with ETT (Figure 6). Michiels Ten Kate and van Vliet recognized ETT in ET and PV are very likely caused by hypersensitive thrombocythemia platelets, which spontaneously activate at high hear in the endarterial circulation and secrete their products thus forming aggregates that transiently plug the microcirculation or result in occlusive platelet thrombi in arterioles or small arteries (Figure 4) [48,49]. Michiels et al. [49] produced clear evidence that erythromelalgia and MIAs in ET and PV patients are relieved (cured) by treatment with aspirin and by reduction of platelet count to normal (<350 × 10^9/L), but not by coumadin and the platelet ADP receptor inhibitor ticlopedin (Figure 5). In patients with thrombocythemia associated with PV, increased hematocrit and whole blood viscosity related to increased interactions of hypersensitive platelets, activated granulocytes (increased LAP-score and erythrocytes) severely aggravate the platelet-mediated microvascular ischemic circulation disturbances into major arterial and venous thrombotic complications (Figure 5) [19,22]. The London PV study group of Wetherly-Mein and Pearson clearly demonstrated in the late 1970s that correction of the hematocrit to normal (0.40) by phlebotomy is associated with significant reduction of major arterial

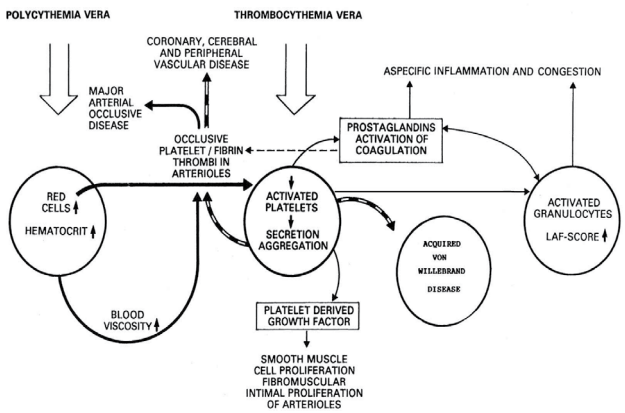

Figure 4: The 1996 concept of Michiels and Van Vliet on the Pathophysiology of aspirin-responsive mirovascular arteriolar inflammation and thrombosis in essential thrombocythemia (ET) and major arterial and venous thrombosis in thrombocythemia polycythemia vera (TPV)as multicellular interaction of hypersensitive platelets, activated leukocytes (increased LAP score) and erythrocytes.

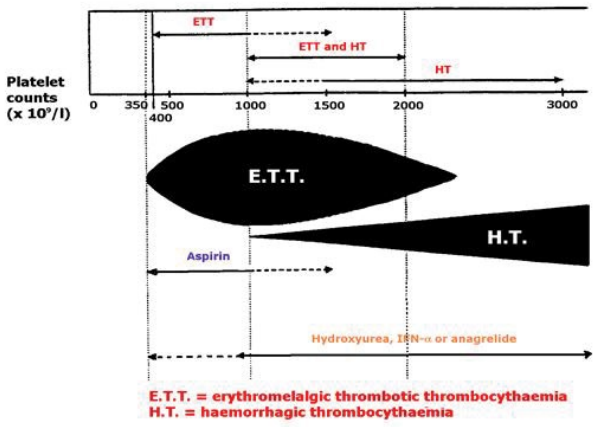

Figure 5: The 1984-1996 concept of platelet-mediated microvascular thrombosis and bleeding in erythromelalgic thrombotic thrombocythemia (ETT) and hemorrhagic thrombocythemia (HT) and the relationship of platelet count in ET and TPV patients according to Michiels Van Vliet and Van Genderen.

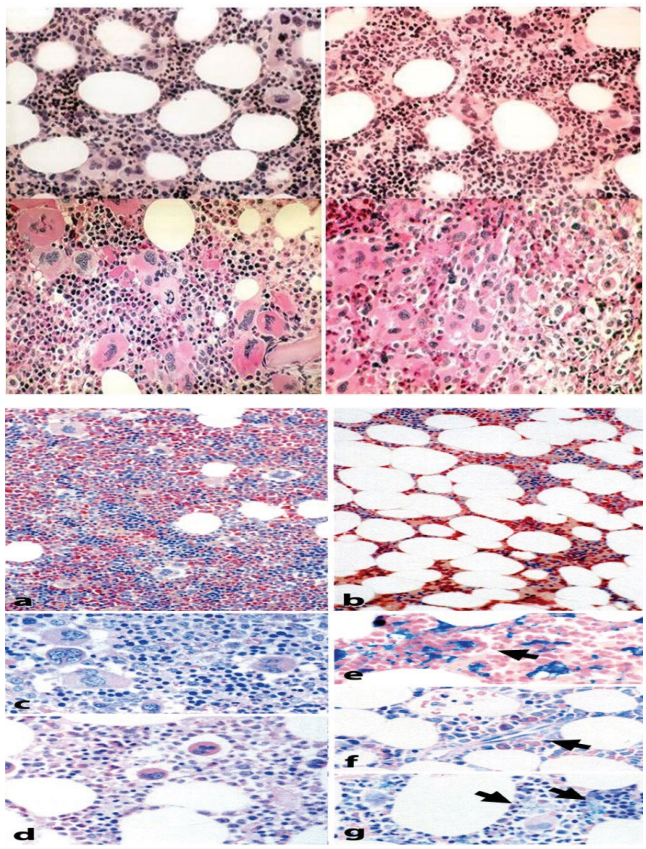

Figure 6: ET (left upper), ET/PV (left right), and PV (left bottum) bone marrow features in ET and PV patients. Pleiomorphic megakaryocytes in ET (upper panels) have similar hyperlobulated nuclei in ET as compared to PV (left bottum). Dense clustered pleiomorphic megakaryocytes in PV/RF (right bottum) in advanced PV show dysmorphic nuclei. From the Pathology Laboratory Dr Ten Kate 1980. Description of bone marrow(BM) histopathology by the expert pathologist Thiele between 1996 and 2002 in polycthemia vera (PV) versus secondary polycythemia (SP). **a** PV with tri-lineage proliferation and hypercellularity including prominent sheets of erythroid precursors and megakaryocytes of diff erent size. **B** SP with slight increase in cellularity in a senescent patient with large islets of erythropoiesis. **c** Pleomorphous aspect of megakaryocytes in PV with sizes ranging from small to large-see also a. **d** Uniform small to medium-sized megakaryocytes in SP. **e** SP with coarse iron deposits in macrophages (arrow). f Prominent perivascular deployment of plasma cells and adjacent eosinophils in SP (arrow). **g** In SP (arrow), deposits of cell debris are regular fi ndings (arrows) (Source, MPD Workshop Rotterdam).

and venous thrombosis, but fails to prevent the platelet-mediated microvascular circulation disturbances because thrombocythemia in PV in remission by phlebotomy alone persisted [18,50]. Michiels et al demonstrated in the 1980s and 1990s that complete relief and prevention of erythromelalgia, atypical microvascular migraine-like transient neurologic and ocular manifestation in ET patients and major thrombosis in PV patients are obtained by treatment with low dose aspirin on top of correction of the hematocrit to 0.40 by phlebotomy, or by treatment with the platelet lowering agents anagrelide or interferon in ET or by reduction of increased platelet and leukocytes by interferon or hydroxyurea in PV in remission by phlebotomy (Figure 5) [20,28,30,48,50,51,52-56].

Van Genderen et al. [55] demonstrated in the 1990s that symptomatic thrombocythemia (ET and PV) patients with erythromelalgic circulation disturbances have shortened platelet survival, increased platelet activation markers beta-thromboglobuline (beta-TG), platelet factor 4 (PF4), increased endothelial cell markers thrombomoduline (TM), and increased urinary thromboxane B2 (TxB2) excretion indicating platelet-mediated processes *in vivo* [52,53]. Inhibition of platelet cyclooxygenase (COX1) by aspirin treatment of thrombocythemia patients complicated by erythromelalgia resulted in the disappearance of erythromelalgic circulation disturbances, correction of shortened platelet survival, and return of plasma levels Beta-TG, PF4, TM and TxB2 excretion to normal [50,52,53]. The Van Genderen studies extended the observations of Michiels and Van Vliet by the demonstration that erythromelalgia and MIAs in ET are characterized by platelet activation and endothelial cell damage but not by thrombin generation, thereby giving a plausible explanation for the inefficacy of coumadin derivatives and heparin in the prevention and treatment of erythromelalgia and MIAs in ET. Histopathology and immunochemical analysis by Van Genderen of skin punch biopsies derived from erythromelalgic skin areas of 2 ET patients showed that erythromelalgic thrombi stained positively with von Willebrand factor (VWF) opposed to a weak fibrin staining [52]. These data produced by Van Genderen that erythromelalgia and MIAs in ET and PV in remission by phlebotomy are caused by platelet-VWF mediated intravascular activation and aggregation of hypersensitive thrombocythemic platelets with subsequent sludging or occlusion by VWF-rich platelet thrombi in the acral, cerebral or coronary microvasculature [23,52].

First-line Treatment for PV with Interferon-alpha (Silver RT and Michiels JJ)

Dr Silver [21] reported on effects of long-term treatment with interferon-alpha for polycythemia vera [38,57]. The most simple and effective treatment option is based on the initial reduction of red blood cells, especially for the elderly is associated with early frequent fatal thrombosis when not on aspirin. Phlebotomy does not diminish the risk of developing myelofibrosis (MF). It has only relatively recently appreciated that hydroxyurea (HU, the standard myelosuppressive drug for PV) is associated with approximately 10% incidence of acute leukemia after long-term follow-up of about 10 years. Additional limitations of using HU in PV include, but are not restricted to, the failure to control constitutional symptoms and toxicity of HU. A review of more than 1200 patients who had PV indicated that the death rate in those receiving P^{32}, alkylating agents or non-alkylating myelosuppressive agents was three to four times higher than those who did not [54]. An increased risk for cancer was observed approximately 6 years after diagnosis of PV suggesting an overall unfavorable effect of myelosuppression.

Silver and Michiels proposed in 1998 to treat PV patients according to the least toxic approach with low dose aspirin and phlebotomy alone in early stage PV and to postpone potential leukemogenic agent hydroxyurea in the intermediate stage of as long as possible by using recombinant interferon (IFN) in the prefibrotic stages of PV [57,58]. If non responsive to IFN or serious side effects there is a clear indication to suppress advanced MPD disease in PV by hydroxyurea. IFN has activities against platelet derived growth factor (PDGF), transforming growth factor beta (TGF-beta). TGF-beta has powerful angiogenic properties. How TGF-beta, PDGF and cytokines interact to cause marrow fibrosis is unknown: what is of importance is that IFN therapy influences TGF-beta values to return to normal levels together with correction of increased platelet counts and spleen size in PV in responsive cases that suggest that IFN has a unique role in the treatment of the MPDs, particularly PV, and may be the first drug which may alter the natural history of the disease PV. Silver stated in 1998 that IFN represents the therapeutic evolution of the PVSG in searching for the best treatment of PV patients. The rational for using IFN-alpha in the treatment of early and intermediate prefibrotic stage of PV according to Silver includes:

1. Abatement of constitutional symptoms including itching
2. Maintenance of hemoglobin, hematocrit, leukocyte in the normal range and platelet suppression.
3. Avoidance of phlebotomy, secondary iron deficiency anemia and secondary macrocytosis associated with hydroxyurea
4. Lacks of mutagenicity.
5. Prevents or delays the development of post-PV splenic myeloid metaplasia and myelofibrosis by reduction of MPD disease burden (reduction splenomegaly).
6. Effectiveness/toxicity (risk/reward) ratio is positive.
7. Because recombinant IFN-alpha is suppressive, not curative, long-term treatment is required with careful attention to the short- and long-term nonhematopoietic effects of IFN-alpha.
8. Additional phlebotomy may be required to maintain the hematocrit in the desired target.
9. For those younger patients who will not accept IFN, phlebotomy plus antiplatelet agent anagrelide may be considered [58].
10. For elderly patients (older than 70 years) hydroxyurea should be considered for treatment.

Clear indications for the start of hydroxyurea in stage 3 PV include uncontrolled platelet count of $>1{,}000\times10^9$/l during aspirin therapy; symptomatic large spleen (splenomegaly); PV-related constitutional symptoms including pruritis; leukocyte count in excess of 25×10^9/l (hypercellular PV); leukoerythroblastic blood picture and signs of myeloid metaplasia and major arterial or venous thromboembolic complications despite eradication of vascular risk [57,58]. The main therapeutic benefits of recombinant interferon-alpha (rIFN-alpha) therapy for PV include induction of hematological remission with elimination or reduction in the need of phlebotomy, control of thrombocytosis and leukocytosis and resolution of classical PV disease associated symptoms in particular refractory pruritis [57]. Reviews of the literature anno 1998 indicate that an overall hematological response has been achieved in more than 75% of cases within 6 to 12 months [38,58]. Doses of rIFN-alpha used to obtain hematological response

ranged from 2 MU per week to 70 MU per week with a median dose of about 10 MU per week. Usually, dose reduction occurred in the second year of treatment [57].

The 1997-2000 Diagnostic Criteria of the MPDs ET, PV and CMGM/PMGM (Michiels JJ)

Philadelphia chromosome-positive (Ph^+-ET) and chronic myeloid leukemia (Ph^+-CML) constitute a separate malignant disease entity whereas ET, PV and chronic or primary megakaryocytic, granulocytic myeloproliferation (CMGM/PMGM) belong to the Ph-negative myeloproliferative disorders (MPDs) [57,58]. The megakaryocytes in Ph^+ ET and CML are abnormal small with round nuclei, showing little lobulation. Both the number and size of megakaryocytes in Ph^-negative ET, PV and CMGM/PMGM are typically increased with loose or dense clustering of large megakaryocytes. Enlarged mature megakaryocytes with multilobulated nuclei and their tendency to cluster in a normal or slightly increased cellular bone marrow represent the hallmark of ET (Table 3 and Figure 6) [58,59]. The size and morphology of megakaryocytes are normal in reactive thrombocytosis, very small in Ph^+ ET and CML and enlarged in all variants of MPD. The characteristic increase and clustering of enlarged mature and pleomorphic megakaryocytes with multilobulated nuclei and proliferation of erythropoiesis in a moderate to marked hypercellular bone marrow (50 to 70%) is the diagnostic hallmark of latent, prodromal and early stage PV (Table 4 and Figure 6) [58-60]. Diagnostic bone marrow histopathology characteristics in classical PV are described by the trias of:

1. Increase of clustered pleomorphic medium sized and large mature megakaryocytes with hyperploid nuclei.
2. Increased cellularity (90 to 100%) due to trilinear erythrocytic, megakaryocytic and granulocytic (EMG) myeloproliferation.
3. No or minor increase of fine reticuline fibers (Table 4 and Figure 6) [58-60].

In secondary polycythemia in which increased cellularity of the erythroid cell line may be present the number, size and morphology of megakaryocytes remain normal (Figure 6). The early prefibrotic stages stages of AMM or prePMF (Table 5, Figures 7 and 8) and fibrotic stages of AMM or PMF with anemia and large spleens without any PV features according to the PVSG appears to be a distinct dual chronic or primary megakaryocytic granulocytic myeloproliferation (CMGM/PMGM) in the Hannover Bone Marrow Classification of the MPDs [56,57]. The histopathology of the bone marrow in PMGM is dominated by atypical enlarged (larger than in ET and PV) and immature megakaryocytes

Diagnostic	
A1	Platelet count in excess of 400 × 10^9/L and no known cause of reactive thrombocytosis.
A2	Increase and Clustering of enlargedand mature megakaryocytes with hyperploid nuclei in bone marrow specimens.
A3	No preceding or allied other subtype of myeloproliferative disorder or myelodysplastic syndrome.
Confirmative	
B1	Normal or elevated leukocyte alkaline phosphatase score,normal ESR, and no fever or infection.
B2	Normal or increased cellularity of bone marrow with or without the presence of reticulin fibres in biopsy material.
B3	Splenomegaly on palpation or diagnostic imaging.
B4	Spontaneous erythroid colony formation and/or spontaneous megakaryocyte colony formation in bone marrow culture.

Table 3: The 1980 and 1997 Rotterdam Clinical and Pathological (RCP) criteria for the diagnosis of Essential Thromocythemia proposed by Michiels & Juvonen.

Diagnostic Criteria PV		Confirmative Criteria PV	
A1	Raised red cell mass: RCM male>36 ml/kg, Female>32 ml/kg	**B1**	Thrombocythemia platelet count>400 × 10^9/L
A2	Absence of any cause of secondary erythrocytosis by clinical and laboratory investigations	**B2**	Granulocytes>10 × 10^9/L and/ or raised LAP score in the absence of fever or infection
A3	Histopathology of bone marrow biopsy: (a) Increase and clusters of pleomorphic enlarged megakaryocytes with hyperploid nuclei (b) Increased cellularity: panmyelosis (c) Reticulin fibres (optional)	**B3**	Spontaneous erythroid colony formation in the absence of EPO and low plasma EPO level
A1+A2+A3 is consistent with early plethoric stage of PV ("idiopathic erythrocytosis") *A2+A3+elevated haematocrit>0.05 is consistent with latent or early plethoric stage PV* *A1+A2+A3+any one from category B establish overt PV according to PVSG* *A2+A3+elevated hematocrit>0.05+any one feature in category B plus a typical clinical picture establish overt PV without the need of red cell mass measurement.* *A3+B1 are consistent with essential thrombocythemia with features of latent PV.* *A3+B3 and/or B4 is consistent with latent PV or primary MPD*			

Table 4: The 2000 European Clinical and Pathological (ECP) criteria for the detection of latent, prodromal, erythrocythemic, classical and advanced stages of Polycythemia Vera by including bone marrow histopathology according to Michiels et al.

with cloud-like nuclei which are not seen in Ph-ET and PV and Ph^+ ET and CML (Table 5 and Figures 7 and 8) [52-57]. Myelofibrosis in ET, PV and CMGM is graded in no reticulin fibrosis (MF 0), early reticulin fibrosis (MF 1), advanced reticulin fibrosis with minor collagen fibrosis (MF 2) and advanced collagen fibrosis with or without osteosclerosis (MF 3) [25,61-65].

Bone Marrow Histopathology(Thiele J and Kvasnicka HM)

Thiele & Kvasnicka presented a systematic study on the modem approaches towards bone marrow histopathology and immunohistochemistry as a specific clue to diagnose and stage (Table 5) the myeloproliferative disorders (MPD) ET, PV and CMGM/PMGM) [64], and to differentiate PMGM from ET and PV based on clinicopathological criteria (Table 5, Figures 7 and 8) [61-63]. The morphological approach attempts to assess the different subtypes of MPDs by regarding their most prominent histological features like megakaryocytes and associated reticulin-collagen fibers, and myeloid or erythroid precursors. This methodology according to Michiels & Thiele will become most successful by the use of enzyme- and immunohistochemistry with a meticulous conducted analysis of megakaryo-erythro-granulopoiesis and grading of reticulin and collagen fibrosis, which has to be followed by correlation of the relevant laboratory and clinical parameters for proper staging of each of the MPDs ET, PV and CMGM/PMGM (Table 5 and Figures 7 and 8). PV is not only characterized by a striking increase and clustering of pleomorphic large megakaryocytes. This clustering consists of a grouping of giant megakaryocyte together with medium-to small-sized and degenerative cell forms or naked nuclei (Figure 7). No major megakaryocyte maturation defects are observable throughout the bone marrow. As in the other subtypes of MPDs there is an obvious dislocation of megakaryocytes from the center of the bone marrow to the paratrabecular endosteal area. The number and sizes of large and mature megakaryocytes are significantly increased in ET, to large but histology fails to display maturation disturbances seen in PMGM (Table 5 and Figure 8). It should be emphasized that giant mature megakaryocytes with hyperlobulated nuclei in a normocellular bone

European Clinical and Pathological (ECP) criteria of ET by including bone marrow histopathology according to Michiels, Georgii and Thiele for ET published in the Netherland Journal of Medicine 1999.			
European Clinical and Pathological (ECP) criteria of ET according to Michiels 1997 and Michiels & Thiele 2002			
A1	Platelet count in excess of 400 × 10^9/L and no known cause of reactive thrombocytosis.		
A2	Increase and clusters of mature giant megakaryocytes with hyperploid nuclei in bone marrow biopsies.		
A3	No preceding or allied other subtype of myeloproliferative disorders or myelodysplastic syndrome.		
Confirmative			
B1	Normal or elevated leukocyte alkaline phosphatase (LAP) score, normal ES, and no fever		
B2	Normal or slightly increased cellularity and no or minimal reticulin fibrosis in bone marrow biopsies		
B3	Splenomegaly on palpation, or >11 cm on ultrasound scan or on computer tomogram (CT).		
B4	Spontaneous erythroid colony (EEC) and/or spontaneous megakaryocyte colony formation (CFU-Meg).		
European Clinical and Pathological (ECP) criteria of PV by including bone marrow histopathology according to Michiels et al.			
Diagnostic criteria		**Confirmative criteria**	
A1	Raised red cell mass: RCM, erythrocytes >6 × 10^{12}/Lmale >36 ml/kg, female >32 ml/kg	**B1**	Thrombocythemia platelet count >400 × 10^9/L
A2	Absence of any cause of secondary erythrocytosis by clinical and laboratory investigations	**B2**	Granulocytes >10 × 10^9/L and/or raised LAP score in the absence of fever or infection
A3	Histopathology of bone marrow biopsy: a) increase and clusters of pleiomorphic megakaryocytes with hyperploid nuclei b) increased cellularity: panmyelosis c) reticulin fibers (optional)	**B3**	Splenomegaly on palpation or >11 cm on ultrasound scan or CT
		B4	Spontaneous erythroid colony formation in the absence of Epo and low plasma Epo level
European Clinical and Pathological (ECP) criteria of CMGM/PMGM by including the Hannover Bone Marrow Classification proposed by Georgii (1990,1996), Michiels (1997) and Michiels & Thiele			
Clinical and hematological features Diagnostic criteria of CMGM/PMGM Georgii 1996, Michiels 1997			
A	No preceding subtype of ET PV CML or MDS	CMGM/PMGM: Chronic or primary megakaryocytic granulocytic myeloproliferation (CMGM/PMGM) according to Georgii & Michiels	
B	Thrombocythemia, platelets from >400×10^9/l to high around and above 1000 × 10^9/L	Abnormal clustering and increase of atypical giant to large megakaryocytes atypical giant to large megakaryocytes and definitive maturation defects of cytoplasm and cloud-like nuclei (Georgii 1990)	
C	Splenomegaly normal (<12 cm) to slight increase (<15 cm) to large >(<18 cm) on ultrasound scan or CT	Myelofibrosis MF: criteria Georgii 1998 MF 0. no reticulin fibrosis=prefibrotic MF 1. slight reticulin fibrosis=early fibrotic	
AMM or classical PMF			
D	Anemia, hemoglobine <12 g/dl	MF 2. marked increase (density) in reticulin and/or collagen fibrosis= fibrotic	
E	Definitive leuko-erythroblastic blood picture and/or tear drop erythrocytes	MF 3. advanced collagen fibrosis/osteosclerosi endophytic bone formation= advanced fibrotic	
Clinicopathological staging of (PMGM) Michiels & Thiele			
Stage 0	A+B ± C	+MF_0 is consistent with a hypercellular prefibrotic stage of EMGM simulating ET.	
Stage 1	A+B+C ± D	+MF_1 is consistent with early PMGM/MF or MF.	
Stage 2	A+B+C ± D	+MF_2 is consistent with manifest MF or AMM.	
Stage 3	A+B+C ± D	+MF_2+$_3$ is consistent with advanced or AMM complicated by osteosclerosis, osteomyelosclerosis.	

Table 5: European Clinical and Pathological (ECP) criteria of ET by including bone marrow histopathology according to Michiels, Georgii and Thiele for ET published in the Netherland Journal of Medicine 1999.

marrow are the hallmark of ET. PMGM usually presents with high platelet counts around 1000 × 10^9/L and a hypercellular bone marrow due to pronounced PMGM (Figure 8). The red cell lineage in PMGM appears to be not significantly altered or may be relatively reduced. Prefibrotic PMGM with hypercellularity composed of prominent granulocytic and megakaryocytic proliferation revealing dense clusters of small to giant megakaryocytes with hypolobulated (bulbous) nuclei (Figure 8).

Prognostic Factors in PMGM and Classical Myelofibrosis (MF) (Michiels JJ)

Current diagnostic features of AMM or PMGM as the third MPD entity include prefibrotic stages, fibrotic stages with various degrees of anemia, splenomegaly, leuko-erythroblastosis blood picture, tear drop erythrocytes and various degrees of reticulin and collagen fibrosis in the bone marrow: myelofibrosis reticulin (MF) [25,57,61-64]. Different set of prognostic factors have been proposed. One set of prognostic factors is statistically derived from one series of retrospectively studied MF patients but not applicable to another large series of mF patients. These conflicting results on prognostic factors in MF (Lille, Barosi, Reilly, Hasselbalch) can be explained by differences in diagnostic criteria and lack of uniform criteria for prognosis prediction in MF [57]. This makes selection of MF patients for new therapeutic strategies very problematic. However, all MF studies agree on the Rotterdam clinical scoring system for prefibrotic, fibrotic and advanced fibrotic stages of EMGM/PMGM patients (Table 6). According to PVSG criteria, the early hypercelllar prefibrotic stages of PMGM or PMF (prePMF according to Thiele) are neglected and overlooked in all the MF studies. Based on an elegant series of clinicopathological studies on PMGM or MF by the German pathologists Georgii and Thiele in collaboration with the Dutch hematologist Michiels introduced in 1998 the concept of the European, Clinical and Pathological (ECP) criteria for the diagnosis of three distinct MPD entities of ET, PV and PMGM [25,57,65].

MPN Disease Burden and Therapeutic Options in ET, PV and MF 2000 and Beyond

With the advent of molecular screening of MPN, patients with increased MPN disease burden associated with significant leukocytosis, thrombocytosis, consitutional moderate splenomegaly are candidates for low dose peglyated interferon (PegasysR, 45 mg/mL once per week or every two weeks) as the first line myeloreductive treatment option in

JAK2^{V617F} mutated MPN disease in ET and PV patients [66,67]. A non-responsive to or side effect induced by IFN, hydroxyurea is the second line myelosuppressive treatment option in JAK2^{V617F} mutated ET and PV patients with increased MPN-T disease burden [67]. The top 20 complaints at time of diagnosis in 399 out of 497 (81%) an MPN patient was fatigue (81%) equally high in ET, PV and MF patients [68]. Apart from fatigue about 40% to 60% of ET and PV patients presented with aspirin responsive microvascular disturbances. Itching (PV 58% *vs.* ET 30%) and fatigue were much more prominent in PV. About one third of MPN (ET, PV and MF) patients suffered from bone pain. MF patients suffered more frequently from constitutional symptoms of prominent fatigue and night sweats related to pronounced splenomegaly. Before the diagnosis was made in 497 MPN patients, the complaints were ascribed to other causes in 173 (35%): to stress, burned out or overstrained in 41 (24%), to depression or hystery in 14 (8%), migraine of unknown origin in 13 (8%) and to rheuma, hypertension or fibromalgia in a few (Figure 9) [68].

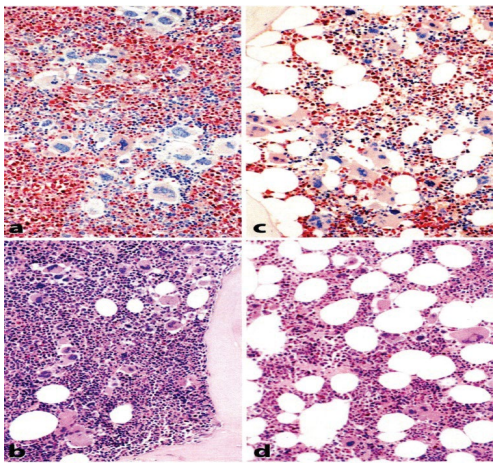

Figure 7: Description of bone marrow (BM) histopathology by the expert pathologist Thiele between 1996 and 2002 in prefibrotic hypercellular primary megakakaryocytic granulocytic myeloproliferation (PMGM) versus normocellular essential thrombocythemia (ET). **a** Prefibrotic PMGM with hypercellularity composed of prominent granulocytic and megakaryocytic proliferation revealing dense clusters of small to giant megakaryocytes with hypolobulated (bulbous) nuclei. **b** In routinely stained specimens clustering and abnormal endosteal translocation of megakaryocytes and the prominent granulopoiesis are easily recognizable. **c** ET with age-matched cellularity and a predominant proliferation of large to giant mature megakaryocytes showing extensively lobulated nuclei and a lack of signifi cant proliferation or left-shifting of the other cell lineages. **d** Dispersed or loosely clustered large to giant mature megakaryocytes are the diagnostic hallmark of ET. (Source MPD Workshop Rotterdam).

Outlook 2000 and Beyond

Clinical, hematological and morphological peripheral blood and bone marrow features in particular, megakaryocyte morphology and bone marrow cellularity according to Georgii and Thiele, reveal diagnostic clues and pathognomonic features, which enables a clear-cut distinction between ET, PV and PMGM. Increase of clustered large mature megakaryocytes with mature cytoplasm in a normal or slightly increased erythropoiesis represents the hall mark of ET (Figure 6). Increase and clustering of large mature and pleiomorphic megakaryocytes with multilobulated nuclei and increase of moderate erythro-megakaryocytic (EM) hyperplasia to marked trilinear erythro-megakaryo-granulocytic hypercellular bone marrow with dilated sinuses are the specific diagnostic features of early and classical untreated PV (Figures 6 and 7). ET may precede PV for many years to more than a decade. Prefibrotic EMGM/PMGM (Tables 5 and 6) is a distinct MPD without features of PV in blood and bone marrow (Figure 8). The

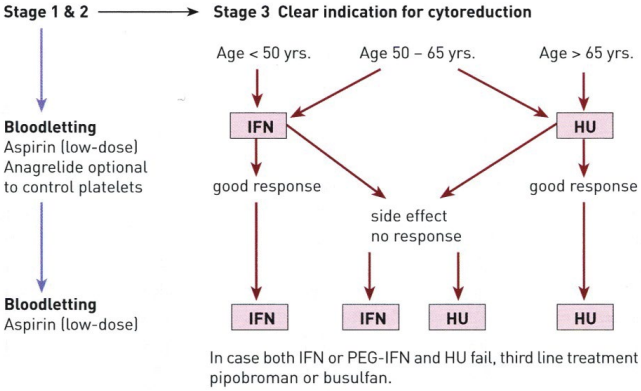

Figure 8: Study design of the european collaboration on low dose as in polycythemia vera (ECLAP): An observational study in pv with a clear indication of aspirin in pv and a randomized clinical trial in pv not on aspirin. The ECLAP study has been designed by landolfi, michiels and patrono between 1994 and 1997 on behave of the scientific working group (SWG) on MPD within the european hematology association (EHA).

Prognosis Group	Criteria	Score	Clinical Stage
Very favorable	Hemoglobin normal >12 g/dl, no anemia. No fibrosis of the bone marrow: EMGM	Score 0	Very early
Favorable	Hemoglobin >10 g/dl, slight anemia	Score 1	Early
Intermediate	Hemoglobin >10 g/dl, slight anemia, but no othct adverse sign*	Score 2	Over
Unfavourable	Two adverse signs*	Score 3	Advanced
Very Unfavourable	More than 2 adverse signs*	Score 4	Endstage
Adverse signs*	Hemoglobin <l0 g/dl, blast PB >I %, precursors WBC. in PB >10%, serum LDH >3 times upper limit, leukopenia <3, leukocytose >20 or >30, thrombocytopenia <100×N 10^9/l constitutional symptoms, massive splenomegaly, cytogenetic abnormality.		
Rotterdam scoring system of EMGM/IMF			
1.	A hemoglobin >12 mmol/l and no adverse signs predicts a favorable prognosis, score 0.		
2.	A hemoglobin <10 mmol/l and not adverse signs predict a favorable prognosis, score 1.		
3.	A hemoglobin <10 g/dl, but absence of any other adverse sign was associated win a significantly shortened survival in all studies, intermediate prognosis, score 2.		
4.	The presence of two of the above mentioned adverse signs predicts a poor prognosis, score 3.		
5.	The presence of more than two of the above mentioned adverse signs predicts a very poor prognosis, score 4.		

Table 6: Clinical and laboratory characteristics for prognosis prediction in essential or primary megakaryocytic granulocytic myeloproliferation EMGM/PMGM (IMF) patients according to the 1999 rotterdam scoring system.

histopathology of EMGM/PMGM is dominated by atypical large and immature megakaryocytes with cloud-like immature nuclei, which are not seen in prefibrotic ET and PV at diagnosis and during follow-up. Myelofibrosis is not a feature of ET at diagnosis and during long-term follow-up. Myelofibrosis, which is secondary to myeloid metaplasia of bone marrow proliferation (myeloproliferation), and myeloid metaplasia of the spleen (splenomegaly) constitute a prominent feature and usually progress more or less rapidly during the natural history of PV and PMGM. Life expectancy is normal in normocellular ET. Normal in the early stages of prodromal and classical stages of PV, but significantly shortened in the thrombocythemic hypercellular EMG stage of PV in the various fibrotic stages of PMGM. These clinical and pathological characteristics of the MPDs, by including bone marrow histopathology, enables clinicians and pathologists to clear-cut distinct between normocellular ET, prodromal PV, classical PV hypercellular PV *vs.* the prefibrotic and fibrotic stages of EMGM/PMGM.

The ECP manuscript on diagnosis and treatment of PV and possible future study designs of the International PVSG by Michiels, Barbui, Finazzi, Fruchtman, Kutti, Rain, Silver, Tefferi and Thiele in 2000 describes clinicopathological criteria to distinct the six sequential stages proposed by Wasserman and Michiels (Figure 2 and Tables 6 and 7) in the natural history of newly diagnosed PV patients. The European Working Group on MPD (EWG.MPD founded and chaired by Michiels 1998-2006) extended and modified the PVSG diagnostic criteria of ET, PV and PMF into normocellular ET at platelet count above 400×10^9/L, five stages of PV and PMGM as the third MPD entity by including bone marrow histology 1975-2005. From the results of the USA and French PVSG prospective randomized clinical trials in PV showing leukemogenicity of P^{32} and chlorambucil as compared to phlebotomy, it became evident at the first International MPD Workshop in Rotterdam (1998) that new prospective randomized clinical trials (RCT) are warranted in previously untreated early classical and hypercellular stages of PV. Such RCTs should focus on comparing interferon-alfa, a non leukemogenic myeloreductive biological modifier versus a potential leukemogenic myelosupprevive treatment modality. Hydroxyurea appeared to be the least leukemogenic myelosuppressive agent as compared to P^{32} and Busulfan in long-term prospective clinical PV-studies extending treatment observation periods of more than 10

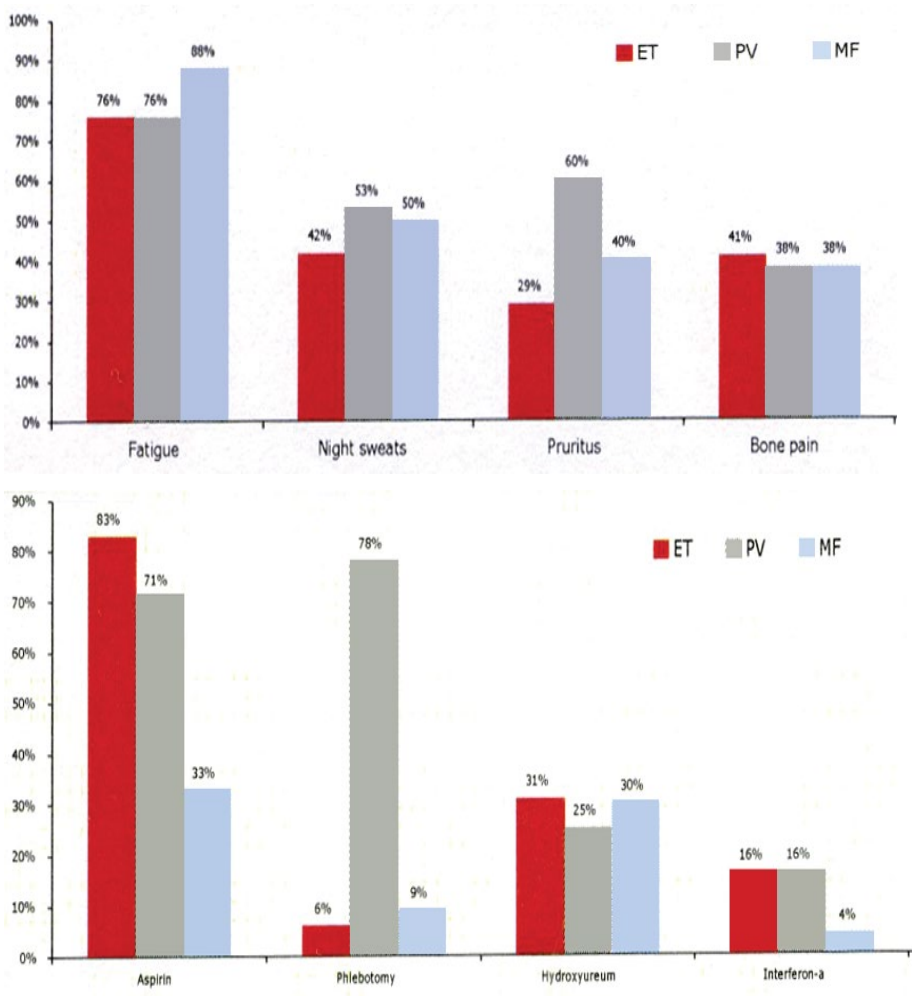

Figure 9: Survey of 363 myeloproliferative neoplasm (123 essential thrombocythemia, ET, 190 polycythemia vera, PV and 50 myelofibrosis, MF) patients: 93% of PV, 71% of ET and 37% of MF were on aspirin; 6% of ET, 78% of PV and 9% of MF were treated with phlebotomy. Because of symptomatic MNP disease burden 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).

Stage	0ET	1PV	2PV	3PV PV MF	4	5 Spent Phase
Hemoglobin (mmol/l)	N/↑	↑	↑	↑	↑	N/↑
Erythrocytes >6 × 10^{12}/l	N/↑	↑	↑	↑	↑	N/↑
Red cell mass:						
Male>36ml/kg	N	↑	↑	↑	↑	
Female>32 ml/kg	N	↑	↑	↑	↑	
Hematocrit	N/↑	↑	↑	↑	↑	N/↑
Platelets × 10^9/l	<400/>400	<400	>400	>1000	Variable	N/↑
Leukocytes × 10^9/l						
Leukoerythroblastosis	-	-	-	±		
Spleen lengh diameter (cm) on scan or echogram	<12 12-15	<12	<15	>15	Large	
Bone Marrow Biopsy						
Cellularity	N/↑	N/↑	↑↑	↑↑	↑↑	↑↑
Megakaryocytes	↑	↑	↑↑	↑↑	↑↑	↑
Myelofibrosis	0	0	0/1	1/2	2/3	3
Spontaneous EEC	+	+	+	+	+	+
PVR 1-gene	+	+	+	+	+	+

N=Normal, =Absent, +=Present, ↑=increased, ↑↑=Pronounced increased.
Six clinicopathological stages of PV according to Wasserman and Michieles: **0:** latent or subclinical prefibrotic PV; **1:** the so called erythemia of Wasserman the so called idiopathic erythrocytisis of Pearson; **2:** early stage PV with no significant or slight splenomegaly and no or early MF; **3:** overt plethoric stage of PV with various degrees of pronounced splenomegaly and MF; **4:** advanced myelofibrotic or spent phase PV; **5:** transformation to MDS and/or AML.

Table 7: Six stages of ET→PV according to Wasserman and Michiels.

years. The rational for using pegylated interferon (Intron or Pegasys) as a first-line treatment option in newly diagnosed PV patients include its effectiveness to abate constitutional symptoms and to induce complete hematological remission thereby avoiding phlebotomy, iron deficiency, and microcytosis associated with hydroxyrea. Moreover, pegylated IFN-alpha may prevent or delay the development of myelofibrosis if used early and classical stage of previously untreated PV [60]. Clinicians will be reluctant to postpone or delete the use of hydroxyurea early in the course of PV as long as low dose aspirin on top of phlebotomy aiming at a hematocrit of 0.40 is used to keep the PV patient as healthy as possible [51,60]. Low dose aspirin will prevent the microvascular thrombotic complications in ET and in thrombocythemia associated with PV in remission after phlebotomy but lacks myeloreductive and suppressive activity [58,67-69]. Selective control of megakaryocyte maturation will predict a significant reduction of microvascular complications in ET and early stages of prodromal and classical PV and reduction of platelet production to normal (400 × 10^9/L in ET and <350 × 10^9/L in PV by relatively low doses of anagrelide [60]. It took more than 20 years before the MPD criteria for ET, PV and prefibrotic AMM=prefibrotic PMF=prefibrotic PMGM as defined by Michiels and Thiele beween 1998 and 2004 (Tables 3-5) [64-66] are included as major criteria in the 2006 ECMP and the 2015 WHO-ECMP criteria defined by Michiels et al. for the trilinear MPN JAK2^{V617F} ET and PV versus CALR or MPL thrombocythemia and myelofibrosis [69].

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