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Bone Marrow Features and Natural History of BCR/ABL-Positive Thrombocythemia and Chronic Myeloid Leukemia Compared to BCR/ABL-Negative Thrombocythemia in Essential Thrombocythemia and Polycythemia Vera

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Abstract

The Hannover bone marrow (BM) classification distinguished three phenotypes of BCR/ABL-positive CML: CML of common type (CML.CT), CML with megakaryocyte increase (CML.MI) and CML with megakaryocyte predominance (CML.MP). BCR/ABL-positive essential thrombocythemia (Ph-positive ET) is featured by CML.MP bone marrow picture of small monolobulated megakaryocytes and is part of the CML spectrum as a malignant disease (neoplasia) with an obligate transition into acute leukemia of near to 100% after 10 years follow-up. The Hannover BM classification distinguished three primary prefibrotic BCR/ABL-negative (Ph-negative) myeloproiferative disorders (MPD)s: essential thrombocythemia (ET), polycythemia vera (PV) and chronic or primary megakaryocytic granulocytic myeloproliferation (CMGM/PMGM). The incidence of blasts crisis is low in the Ph-negative MPDs ET, PV and CMGM. The risk of myelofibrosis is high in CMGM/PMGM, moderate in PV but low in Ph-negative ET. In BCR/ABL-positive thrombocythemia the platelets are small and indolent (non-reactive) and megakaryocytes are smaller than normal with hypolobulated nuclei caused by BCR/ABL induced maturation defect. BCR/ABL-positive thrombocythemia does not present erythromelalgic thrombotic or bleedings manifestations at increased platelet count in excess of 400 to 1500×10^9/L. The platelets and megakaryocytes in BCR/ABL-negative ET and PV are large due to growth advantage caused by constitutively activated by the JAK2V617F or MPL515 mutation. JAK2 and MPL mutated thrombocythemias are associated with a high risk on aspirin responsive platelet-mediated inflammation and thrombosis in the end-arterial circulation (platelet thrombophilia).

Keywords: Philadelphia chromosome; BCR/ABL fusion gene; Chronic myeloid leukemia; Essential thrombocythemia; Polycythemia vera; Myelofibrosis; Neoplasia; Myeloproliferative disorders; Myeloproliferative neoplasms

Introduction

The Philadelphia chromosome is a disease specific marker for CML patients [1]. Rowley discovered that the a large part of chromosome 22q is translocated to 9q resulting in the minute Ph1-chromosome and that a small part of chromosome 9q is translocated to chromosome 22q resulting in the translocation t(9;22) (q34;q11) [2,3]. Heisterkamp. Groffen Grosveld found that c-ABL was translocated to the Ph1-chromosome and subsequently discovered the breakpoint cluster region (BCR) on chromosome 22 [4-7]. The BCR/ABL fusion gene produces a BCR/ABL protein, which has a high tyrosine kinase activity and CML-transformation capacity [8-10]. Ninety percent of all CML patients are Ph1+/BCR/ABL+, 5% are Ph-/BCR/ABL+, and 5% are Ph-/BCR/ABL- , the latter group usually diagnosed as atypical CML, juvenile CML, chronic neutrophilic leukemia or chronic myelomonocytic leukemia [11]. The tyrokinase activity of the BCR/ABL protein is crucial for the leukemic transformation of hematopoietic cells in vitro and in vivo [8-12]. In this report we summarize the clinical presentation and natural history of BCR/ABL-positive CML and natural history in the period before the introduction of imatinib. The development of imatinib as a therapeutic agent for CML became reality in the 21th century changed the prognosis of patients with CML from poor to good [12].

The Hannover Bone Marrow Classification of CML and the MPDS ET, PV and CMGM

BCR/ABL positive CML is a malignant disease with an obligate transition into acute leukemia, whereas the BRC/ABL negative chronic myeloproliferative disorder (MPD) are featured by a benign neoproliferation of one, two or the three hematopoietic cell lines with a normal or near normal survival in the initial 10 to 15 years of follow-up [13]. Michiels et al and the German pathologists Georgii and Thiele recognized that small mono- or bi-nucleated megakaryocytes are diagnostic for the Ph-positive diseases ET and CML. In contrast, large megakaryocytes with hyper-lobulated nuclei are pathognomonic for Ph-negative essential thrombocythemia (Figures 1 and 2) [13-24]. The Hannover Bone Marrow Classification of CML and MPD regarded myelofibrosis (MF) as a reactive secondary feature of

myeloproliferative disease. The terms agnogenic myeloid metaplasia (AMM), myeloid metaplasia with myelofibrosis (MMM), and chronic idiopathic myelofibrosis (CIMF) and primary myelofibrosis (PMF) lack accuracy because it is applied to both the early prefibrotic hypercellular stage and the advanced fibrotic stages [15,16]. Secondary MF in ET, PV and CMGM is classified as reticulin fibrosis (RF) grade 0 and 1 (=MF 0), RF grade 2 (=MF 1), RF 3 with minor collagen fibrosis (=MF 2) and advanced reticulin-collagen fibrosis (RCF) with or without osteosclerosis (=MF 3) [17,18].
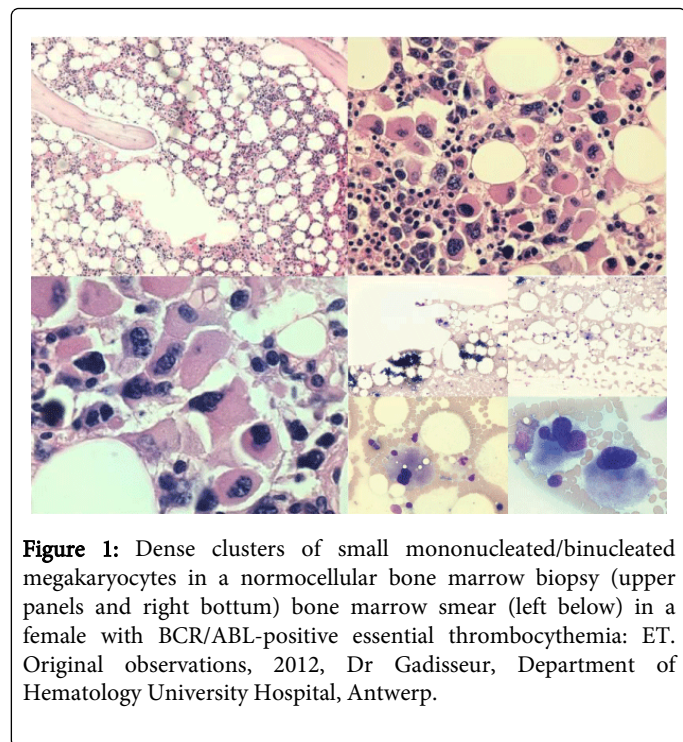

Figure 1: Dense clusters of small mononucleated/binucleated megakaryocytes in a normocellular bone marrow biopsy (upper panels and right bottum) bone marrow smear (left below) in a female with BCR/ABL-positive essential thrombocythemia: ET. Original observations, 2012, Dr Gadisseur, Department of Hematology University Hospital, Antwerp.

In the Hannover Bone Marrow Classification the megakaryocytes in BRC/ABL-positive thrombocythemia and CML are small containing uni- or bi-lobulated nuclei (Figures 1 and 2) but large and mature with hyperlobulated nuclei in ET [16-26]. The megakaryocytes in trilinear PV are of medium size to large (pleomorphic) with hyperploid nuclei. The characteristic increase of clustered enlarged pleomorphic megakaryocytes in a hypercellular bone marrow due to increased erythropoiesis and granulopoiesis (trilinear PV), and no stainable iron are the diagnostic hallmark of PV to distinguish it from congenital and secondary erythrocytoses and reactive thrombocytosis.
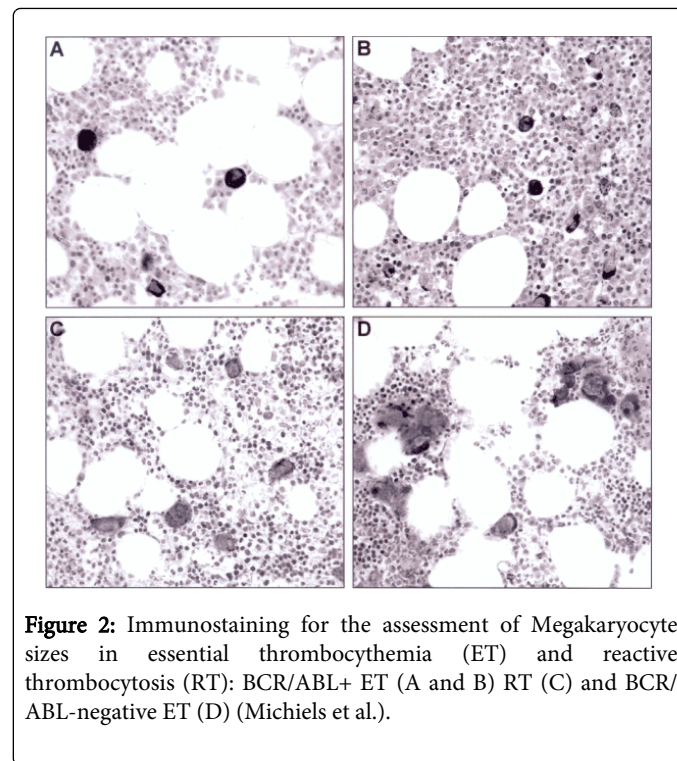

Figure 2: Immunostaining for the assessment of Megakaryocyte sizes in essential thrombocythemia (ET) and reactive thrombocytosis (RT): BCR/ABL+ ET (A and B) RT (C) and BCR/ABL-negative ET (D) (Michiels et al.).

Prefibrotic CMGM is dominated by primary megakaryocytic-granulocytic myeloproliferation (PMGM) and increase of clustered atypical dysmorphic megakaryocytes due to increases of cellular and nuclear sizes. In CMGM/PMGM, the nuclei of dysmorphic megakaryocytes are bulky with clumsy lobuli and irregular roundish shaped form (so-called cloud-like nuclei), which are never seen in ET, PV and CML [16-26]. CMGM/PMGM is not preceded or followed by normocellular ET, PV or MDS and can be regarded as the third distinct prefibrotic primary MPD (Table 1) [19-29].

WHO	Textbooks	Hannover Classification	Percentage (%)
CML and Subtypes	CML or CGL and Subtypes	Primary diseases CML CML.CT CML.M	13.9
P.VERA	P.VERA	P.VERA	17
Thrombocythemia	Thrombocythemia	Thrombocythemia CMGM Pre-early fibrotic	7.1 16
Agno genic myeloid metaplasia (AMM)	Primary idiopathic myelo fibrosis or agno genic myeloid metaplasia	Advanced Disease Increase of blasts Increase of fibres	33.3

Unclassifiable	Unclassifiable	Unclassifiable	12.6
Percentage from 3933-Primary or Idiopathic or Essential Thrombocythemia			

Table 1: The hannover bone marrow classification for CMPD.

Bone Marrow Pathology and Natural History of BCR/ABL+ CML versus BCR/ABL-MPD

Georgii et al. distinguished three bone marrow histology types of BCR/ABL-positive chronic myeloid leukemia (CML): CML of common type with a predominance of granulopoiesis (CML.CT), CML with megakaryocyte increase (CML.MI), and CML with megakaryocytes predominance (CML.MP (Figure 3) [16]. CML-MP is rare and usually seen in Ph-positeve ET without features of CML in blood and bone marrow. The incidence frequency ratio of CML.CT versus CML.MI is 3:1. There are differences between CML.CT and CML.MI in their hematologic features as well as in the clinical course. Significant differences were found in platelet counts, which were more than 2-fold higher in CML.MI as compared to CML.CT (574 versus 257×10^9/L). Both reticulin fibrosis (RF) and advanced reticulin-collagen fibrosis (RCF) occurs more frequently in CML.MI than in CML.CT (49% vs. 14%) in 549 diagnostic bone marrow biopsies collected between 1977 and 1987 (Table 2) [18]. Life expectancy did not significantly differ among the subtypes of CML.CT and CML.M. Patients with CML.M with megakaryocyte increase (MI) grade 2 and 3 have a higher risk to develop myelofibrosis as compared to CML.CT with CML.MI grade 0 or 1 (Figure 4) [18].

Primary Disease (1998)	CML.CT	CML.M	Hannover
Number	415	134	Classification MF
	86%	49%	MF 0=RF 0/1
	7%	27%	MF 1=RF 2
	2%	20%	MF 2 3=RCF ¾
RCF=recticulin andcollagen fibrosis			

Table 2: Grading of (reticulin fibrosis (RF) and reticulin-collagen fibrosis (RCF) and Hannover classification of myelofibrosis (MF) in 549 diagnostic bone marrows from patients with Ph1+ CML.CT and CML.M collected between 1977-1987 (Georgii et al. [16]).
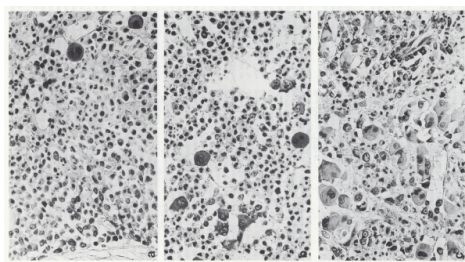

Figure 3: Three main groups of Ph-positive chronic myeloid leukemia (CML): Left, CML common type, CML-CT disturbance in granulopiesis and small dark nucleated megakaryocytes. Middle CML with megakaryocytes increase, CML-MI. Right, CML with megakaryocyte predominance, CML-MP, usually seen in Ph-positive essential thrombocythemia without features of CML in blood and bone marrow.
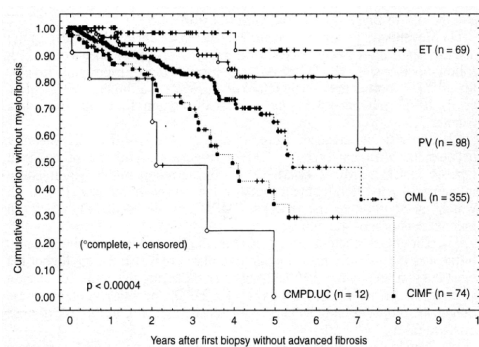

Figure 4: The risk of progress in prefibrotic CML into advanced myelofibrosis grade 2or 3 in 355 follow-up biopsies after up to 8 years is correlated with the grade of megakaryocyte increase (MI)) in the bone marrow (no=MI 0, minor=MI 1, intermediate MI 2 and pronounced MI 3) (Georgii et al.) [16]. Results are based on sequential bone marrow biopsies during long-term follow-up.

In the large Hannover study of 1223 CML, 939 ET, 889 PV and 1026 CIMF/CMGM patients myelofibrosis (MF) grade 1 to 3 in diagnostic bone marrow biopsies was 20% in CML, 3% in ET, 12% in PV and 40% in CMGM/CIMF (Table 3) [17]. The development of MF grade 0/1 into grade 2 or 3 in sequential bone marrow biopsies at 7 years after the first diagnostic bone marrow was 10% for ET, 20% for

PV and 70% for CMGM/CIMF (Figure 5) [17]. Georgii compared the natural history of BCR/ABL-positive CML with BRCR/ABL-negative-prefibrotic CMGM/PMGM, (labeled as CIMF in the 2001 WHO or primary myelofibrosis, PMF in the 2008 WHO classification) as detected by blast crisis outcome studies in follow-up bone marrow biopsies [17,18].

Increase of:	CML	P.vera	ET	CMGM or CIMF
Cellularity (increase)	Evident	moderate	Normal	moderate
Granulopoiesis (increase)	Evident	Slight	Normal	Moderate
Blast excess (EB 2-3)				
Basophils	8.30%	0.40%	0.20%	1.70%
Eiosinophils	Increased	Normal	Normal	Normal
	increased	varying	increased	varying
Erythropoiesis	reduced	increased	normal	Reduced or increased
Iron storage	reduced	negative	normal	reduced
Megakaryopoiesis				
Number of MK	Varying	increased	Increased	Increased
Cellular size	Reduced	increased	increased increased	increased increased
Nuclear size	Reduced	increased	increased	reduced
Nuclear lobulation	Reduced	varying	minimal	prominient
Pleomorphy	Low to evident	moderate		
	varying		moderate	Eminient
Clustering		varying		
Myelofibrosis (MF (1-3))	20%	11.20%	2.90%	40%
Pseudo Gaucher cells	70%	Rare	Unusual	unusual
Lymphoid nodules	Unusual	Evident	Rare	Rare

Table 3: Bone marrow (BM) histological features in early stages of chronic myeloproliferative disorders (MPD) in a total of 4167 diagnostic BM biopsies: chronic myeloid leukemia, CML N=1223, polycythemia vera, P.vera N=889, essential thrombocythemia, ET N= 939 and chronic megakaryocytic granulocytic myeloproliferation, CMGM, or chronic idiopathic myelofibrosis, CIMF N=1026 according to Georgii et al. [17].

In a large 1989-1998 cohort of 1223 CML patients the majority had prefibrotic disease (MF 0 in 78%) and 90% did not have significant excess of blasts in bone marrow biopsies at time of first diagnosis (Table 4) [17]. The incidence of blast crisis (neoplasia) after 8 years follow-up was around 10 to 15% in the cohort of and MPD (ET,PV

and CIMF) patients (N=223) but much higher near to 90% in CML (N=315) (Figure 6) [17], clearly consistent with the clinical observations that that CML indeed is featured by an obligate transformation into acute leukemia within 10 years of follow-up as documented in the German prospective CML management studies (Figure 7) [27].

Number of cases	1323	1323	
Grading MF 199821	Frequency	Grading EB	Frequency
MF-0 No RF	78%	EB 0/1 blasts <20%	90%
MF 1 RF 2	11%	EB 2 Blasts >20%	5%
MF 2 RCF 3/4	4%	EB 3 Blasts >30%	3.3%
MF3 RCS&O	4%	-	-

Table 4: Detection and grading of myelofibrosis (MF) and excess of blasts (EB) in 1323 diagnostic bone marrows from 1323 patients with Ph1+ CML (CML.CT and CML.M) collected between January 1989 and December 1998 (Georgii et al. [17]).

The poor survival curves in CML patients show some advantage in IFN and HU treated as compared to Busulphan treated CML patients (Figure 7). Variables used in the multivariate step-wise analysis in the Cox model included fiber density RF0/1 versus RF 2/3 in bone marrow biopsy and spleen size (but not platelet and leukocyte count hemoglobin or blasts percentage in blood or bone marrow) were used to create 3 risk groups CML patients: risk low n=163, intermediate n=167, high n=165. Such retrospective risk stratification has indeed some impact on prognosis, but none of the CML patients survived after 10 years follow-up [Figure 7] [27].
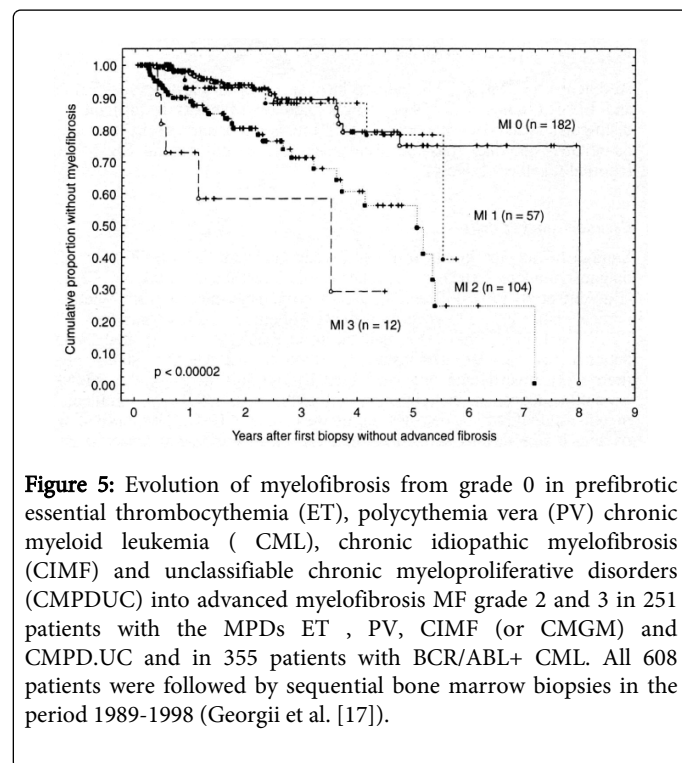

Figure 5: Evolution of myelofibrosis from grade 0 in prefibrotic essential thrombocythemia (ET), polycythemia vera (PV) chronic myeloid leukemia (CML), chronic idiopathic myelofibrosis (CIMF) and unclassifiable chronic myeloproliferative disorders (CMPDUC) into advanced myelofibrosis MF grade 2 and 3 in 251 patients with the MPDs ET , PV, CIMF (or CMGM) and CMPD.UC and in 355 patients with BCR/ABL+ CML. All 608 patients were followed by sequential bone marrow biopsies in the period 1989-1998 (Georgii et al. [17]).
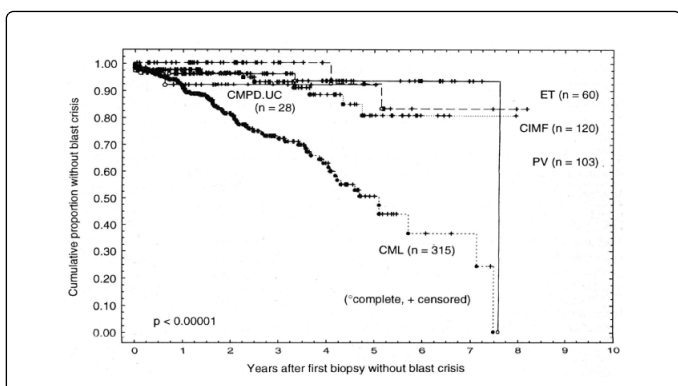
Figure 6: The risk of blasts crisis in 60 ET, 103 PV, 120 CIMF and 315 CML patients as calculated from follow-up biopsies during long-term follow-up of a total of 283 MPD and 315 CML patients in the period 1989-1998. (Georgii et al. [17]) The risk of blasts crisis during a follow-up period of 5to 8 years is low in ET, PV and CIMF but high up to 80% in CML after 7 years of follow-up.
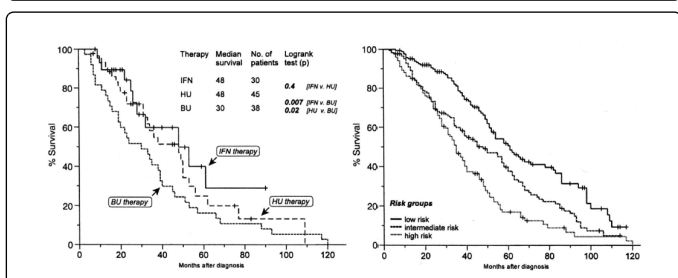

Figure 7: Left. Survival curves in a subgroup of CML patients with manifest myelofibrosis (RF 2/3= RCF) show some advantage in IFN and HU treated as compared to Busulphan treated CML patients. These survival curves in CML clearly demonstrates the obligate transformation of treated CML patients into acute leukemia to near 100% within a mean follow-up of 10 years and confirms the high risk of blastic transformation in the studies of Georgii et al. (Figure 4) [17].Right. Risk stratification according a stratified multivariate Cox analysis show 3 risk groups (low, intermediate and high), but nearly all Ph1-positive CML patients were deceased after 10 years follow-up. Risk low n=163, intermediate n=167, high n=165. Source: Kvasnicka, Thiele, Schmitt-Graeff et al. [27].

Case History of Ph-Positive Essential Thrombocyhemia (ET)

A 47 year old female patient with antecedents of a carcinoma of the ovarium 5 years earlier was transferred to the hematology department of the Antwerp University hospital because of a massive thrombocytosis (>5000 × 10^9/l) in the presence of a normal hemoglobin (16 g/dl) and borderline high leukocyte count (10.6 × 10^6/l) with a normal formula. The JAK-2 mutation proved negative while BCR-ABL was positive. Bone marrow examination showed a myeloproliferative disease with dense clusters of small megakaryocytes and mono-lobulated nuclei consistent with the diagnosis of BCR/ABL-positive ET (Figure 1). Repeated thrombocyte apheresis and hydroxyurea was initiated (Figure 8). After discontinuation of thrombocyte apheresis the thrombocyte count increased again under

hydroxyurea treatment while the leucopenia deepened, and the patient developed a toxic rash. She was then started on Anagrelide (Xagrid®) which resulted in slowly decreasing thrombocyte counts and recuperation of the leucocytes. and a diagnosis of CML was made. Because of development of thrombocytopenia Anagrelide was stopped after 14 days. Imatinib® was started 5 weeks later when thrombocyte counts returned to 4244 × 10^9/l. Under this treatment the thrombocyte count only decreased slowly over a period of 7 months (Figure 8). Patient compliance proved to be an important problem. After these 7 months there was an evolution from chronic phase CML with high thrombocytosis to an acceleration phase/blast crisis with increased leucocyte counts (141 × 10^6/l), appearance of peripheral blasts, and thrombocytopenia (117 × 10^9/l). Imatinib® was switched to second generation TKI (Dasatinib®).
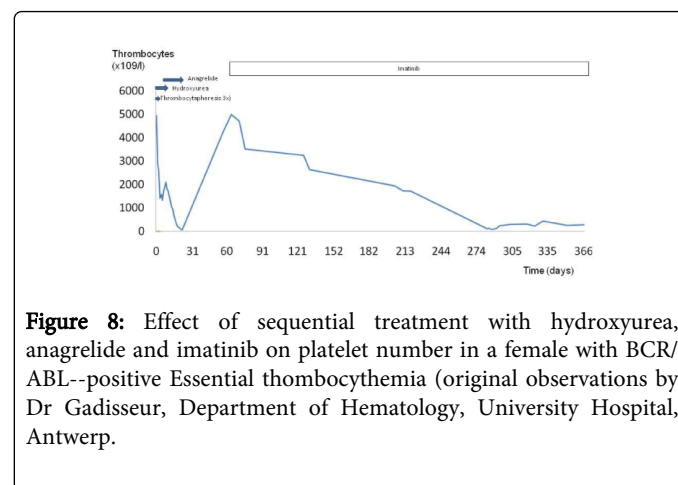

Figure 8: Effect of sequential treatment with hydroxyurea, anagrelide and imatinib on platelet number in a female with BCR/ABL--positive Essential thombocythemia (original observations by Dr Gadisseur, Department of Hematology, University Hospital, Antwerp.

Ph-Positive Essential Thrombocyhemia (ET)

BCR/ABL-positive ET without features of CML in blood and bone marrow is rare but its presentation and natural history is predicted to be similar to CML.MP. The clinical outcome of BCR/ABL positive ET is poor as compared to BRC/ABL-negative thrombocythemia in MPD [14]. The bone marrow in BCR/ABL positive ET is featured by predominant and pronounced mononucleated megakaryopoiesis with initial no, minor or overt granulocytic hypertrophy consistent with CML.MP (Figure 1). Important differences between BCR/ABL-positive ET and BCR/ABL-positive CML at time of presentation are the predilection of BCR/ABL-positive ET for females and the absence of splenomegaly [14]. Ph-positive ET patients have no features of CML in the peripheral blood at time of presentation. Thrombotic or hemorrhagic events are rare in cases of pronounced thrombocytosis associated with Ph-positive CML during follow-up [28,29]. The prognosis of BCR/ABL-positive ET is rather poor prognosis with the development of CML features, and a high risk of progression to myelofibrosis and blast crisis after a few to several years [13,14]. The bone marrow features of the presented case of Ph-positive ET showed that the megakaryocytes in BCR/ABL-positive thrombocythemia are indeed smaller than normal with hypolobulated round nuclei caused by BCR/ABL gene and protein induced maturation defect of the hematopoietic stem cells (Figure 1). This contrasts with clustered enlarged megakaryocytes in BCR/ABL-negative thrombocythemia in various MPDs (Figure 2) [13,14] due to growth advantage and benign proliferation of constitutively activated JAK2 or MPL somatic mutated megakaryocytes [30-40].

Hypersensitive Large Platelets and Megakaryocytes in ET and PV Versus Small and Indolent Platelets and Megakaryocytes in BCR/ABL-Positive Thrombocythemia

Erythromelalgic thrombotic complications (including migraine-like cerebral or ocular ischemic attacks: MIAs) in ET and PV patients are caused by platelet-mediated inflammation and thrombosis in the end-arterial circulation [29-32]. The platelet-mediated erythromelalgic microvascular thrombotic complications are reversible by aspirin and platelet reduction to normal ($<350 \times 10^9$/L), but not by coumadin [31,32].

Erythromelalgic thrombotic complications is rare in BRC/ABL positive CML [28,29]. Despite the high platelet count, the presented case of BCR/ABL-positive thrombocythemia did not present neither erythromelalgic microvascular ischemic events nor bleeding complications. In BCR/ABL-positive thrombocythemia, the small and indolent are non-reactive whereas large platelets in thrombocythemia of various MPNs are hypersensitive with clinical evidence of platelet-mediated erthromelalgic microvascular manifestations in ET and PV patients [31,32].

Recently it became evident that the large platelet are produced by large pleomorphic megakaryocytes in patients with ET and PV carrying the $JAK2^{V617F36}$ or MPL^{515} mutation [34] $JAK2^{V617F}$ or MPL^{515} mutated platelets are constitutively activated and hyper-reactive that can readily explain the high risk of platelet-mediated inflammation and thrombosis in the end-arterial circulation in ET and PV patients [34].

Evidence for hypersensitive activated platelets in thrombocythemia stem from our platelet kinetic studies using autologous platelets labeled with 51Cr-sodiumchromate in symptomatic ET and PV patients as compared to asymptomatic patients with reactive thrombocytosis or thrombocytosis associated with Ph-positive CML (Table 5) [29-31].

Platelet kinetic studies were performed in 3 groups of patients: Group A: 4 healthy controls, 6 asymptomatic thrombocytosis (3 Ph1+ thrombocytosis, 3 reactive thrombocytosis, RT), Group B, 8 with asymptomatic ET or PV, and Group C: 6 with ET or PV complicated by erythromelalgia.

The results are summarized in Table 5 and Figure 9. The linear platelet and disappearance survival curves (A, Figure 9, left) in 6 CML/RT patients was associated with normal platelet survival times indicating that platelets in Ph1+ thrombocythemia and RT and leave the circulation by senescence and no platelet consumption.

The concave platelet survival and disappearance curves (C, Figure 9, right) in 8 thrombocythemia patients (ET or PV) with erythromelalgia (E+) are associated with significantly and pronounced shortened platelet survival time (P=<0.01).

The concave platelet disappearance curves (C, Figure 9, right) demonstrates that ET or PV complicated with erythromelalgia and thrombocythemia indeed is featured by platelet consumption caused by arteriolar platelet-mediated thrombosis as could be seen in skin biopsies from erythromelalgic areas of the same patient [31,32].

The shortened platelet survival times in 4 symptomatic E+ patients could be corrected to normal by aspirin 500 mg/day, which was associated with the disappearance of erythromelalgia clinically as well as in skin biopsy [31,32].

Platelet kinetics: 10^9/L	Platelets`	half life time	mean life	maximal life	platelet	
Patient	N	109/L (R)	Time days	Span days	Span days	turnover
Control	4	138-193	3.9 0.3	6.9 1.6	8.9 0.9	46 20
Group A						
Ph1+CML/ RT	6	722-2244	4.1 0.4	7.8 1.2	8.9 0.9	203 107
Group B						
ET, PV	6	E- 506-1722	3.3 0.3	5.4 0.7	8.0 0.9	335 119
Group C						
ET, PV		8	E+ 489-1130		6.6 0.8	572 374

Table 5: Platelet kinetic studies in Group A: 4 healthy controls, 6 asymptomatic thrombocytosis including 3 Ph1+ thrombocytosis, 3 reactive thrombocytosis, RT), Group B, 8 with asymptomatic ET or PV, and Group C: 6 with ET or PV complicated by erythromelalgia (compare results with Figure 9). N=normal, R=range, RT=reactive thrombocytosis, CML =chronic myeloid leukemia associated with significant thrombocytosis, ET=essential thrombocythemia, PV= polycythemia vera. E-asymptomatic ET and PV. E+ symptomaticET and PV colicatedby erythromelalgia.
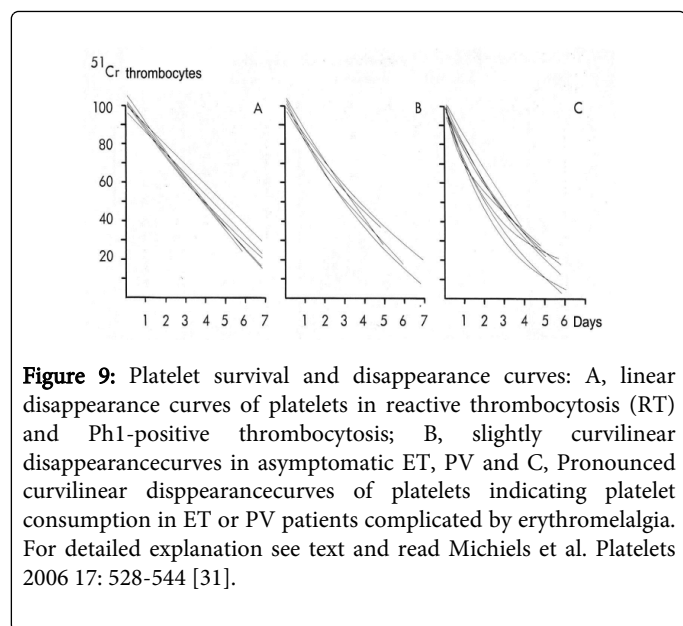
Figure 9: Platelet survival and disappearance curves: A, linear disappearance curves of platelets in reactive thrombocytosis (RT) and Ph1-positive thrombocytosis; B, slightly curvilinear disappearancecurves in asymptomatic ET, PV and C, Pronounced curvilinear disppearancecurves of platelets indicating platelet consumption in ET or PV patients complicated by erythromelalgia. For detailed explanation see text and read Michiels et al. Platelets 2006 17: 528-544 [31].

Discussion

The clinical PVSG and the Hannover bone marrow classification clearly separate the Ph/BCR/ABL-positive ET, CML from the Ph/BCR/ABL-negative MPDs ET, PV and CMGM. The BCR/ABL fusion gene produces a BCR/ABL protein with high tyrokinase activity as the cause of Philadelphia chromsome and BCR/ABL positive thrombocythemia and CML. The BCR/ABL+ neoplasia is a distinct neoplastic entity with a spectrum of early and overt manifestations of the chronic stable and accelerated phases of CML and an obligate transformation into acute leukemia [27]. The Hannover Bone Marrow Classification of CML & MPD [16], distinguished two subtypes of Ph-positive CML, the common type (CML.CT) in which granulocytic hypertrophy is predominant, and CML in which megakaryocytic hypertrophy in addition to granulocytic hypertrophy, in bone marrow biopsy is identified. BCR/ABL+ET is a third variant with no features of CML in peripheral blood, with predominant hypertrophy of small megakaryocytes , and with no, minor or overt granulocytic hypertrophy consistent with CML. BCR/ABL positive ET usually progress to CML, and shows a high tendency to myelofibrosis and blastic transformation [13,14].

The Hannover bone marrow (BM) classification clearly defined ET, PV, CMGM and advanced fibrotic MPD at the bone marrow level [16]. Normocellular ET is featured by the presence of large mature hyperlobulated megakaryocytes [16-18,26,27]. This definition of ET in Table 6 is used in the European Clinical and pathological (ECP) classification (Table 6) [21,22,26,27] and in the 2001 and 2008 classification (WHO-ET) [34-39]. WHO defined bone marrow histology in ET is defined as normocellular (cellularity <60%). WHO defined bone marrow histology in JAK2 mutated PV is hypercellular due to increased erythropoiesis (60-80%) or due to trilinear hematopoiesis (70-100%). Prefibrotic chronic, primary or essential megakaryocytic granulocytic myeloproliferation (CMGM/PMGM) is the third distinct MPD entity without features of PV in blood and bone marrow (Table 6). Since the discovery of the $JAK2^{V617F}$ mutation as the cause of trilinear MPN (PV, ET and MF) Michiels et al. extended the ECP (Table 6) by including $JAK2^{V617F}$ mutation screening in the 2007 WHO-ECMP [37] and in the 2015 WHO-Clinical Molecular and Pathological (WHO-CMP) classifications [39,40]. Good evidence exist that the characteristic features of prefibrotic $JAK2^{V617F}$ mutated trilinear myeloproliferative neoplasms (MPN) can be defined as a broad biological continuum of the earliest benign stage of normocellular ET, ET with features of PV (prodromal PV), polycythemia vera (PV), and hypercellular ET due to essential megakaryocytic granulocytic myeloproliferation (EMGM) when the integrated 2015 WHO-CMP) criteria are applied [39,40]. The $JAK2^{V617F}$ positive MPNs ET, PV and EMGM (either heterozygous, hetero-homozygous, homozygous or "trizygous 9p") represent the classical trilinear MPD with bone marrow features showing a broad spectrum of ET and PV with myeloid metaplasia of the spleen and myelofibrosis during very long-term follow-up [39,40]. A small percentage of JAK2 wild type ET carry the MPL^{515} mutation, which similar to $JAK2^{V617F}$ thrombocythemia is also associated with a high prevalence of erythromelalgic thrombotic complications including MIAs [40]. Recently we discovered that JAK2/MPL wild type ET and MF carrying the CALR mutation is associated with typical PMGM bone marrow characteristics without features of prodromal or overt PV at diagnosis and during life-long follow-up [40].

ET Major criteria	
A1	Persistent platelet count in excess of 400 × 109/L.
A2	Increase and clustering of enlarged mature megakaryocytes in bone marrow biopsy
ET Confirmative criteria	
B1	Presence of large platelets in a peripheral blood smear
B2	Absence of any underlying disease for reactive thrombocytosis and normal ESR.
B3	No or slight splenomegaly on palpation or scan (<15 cm)
ET Exclusion criterion	
Ph+ chromosome or any other cytogenetic abnormality in blood or bone marrow cells	
PV Major criteria	
A1	Raised red cell mass. Male >36 ml/kg, female >32 ml/kg consistent with erythrocytes count above >6 × 1012/L
A2	Absence of primary or secondary erythrocytosis by clinical and laboratory investigation (a typical PV bone marrow excludes erythrocytosis, Kurnick 1972)
A3	Slight, moderate or marked increase in bone marrow biopsy material of: clustered, mature, enlarged pleiomorphic megakaryocytes with hyperlobulated nuclei

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and moderate to marked increase cellularity of megakaryopoiesis/erythropoiesis or typically trilinear mega-erthro-granulopoiesis.

No or presence of reticuline fibers and no collagen fibers (no dry tap)

PV Minor criteria

B1   Thrombocythemia, persistant increase of platelet >400 × 109/L

B2   Leukocytosis, leucocytecount >10×/L and low erythrocyte sedimentation rate (ESR)

B3   Raised leukocyte alkaline phosphatase score >100, absence of fever or infection

B4   Splenomegaly on palpation or on isotope/ultrasound scanning

A1+ A3 plus one of B establishes PV and excludes any variant of erythrocytosis.

Table 6: European Clinical and Pathological (ECP) criteria for the diagnosis of essential thrombocythemia (ET), polycythemia vera (PV), and hypercellular ET due to chronic, primary or essential megakaryocytic granulocytic myeloproliferation.

References

1. Nowel PC, Hungerford (1960) A minute chromosome in human chronic granulocytic leukemia. Science 132: 1497.

2. Rowley J (1973) A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence Giemsa staining. Nature 243: 290-291.

3. Rowley JD (1980) Ph1-positive leukaemia, including chronic myelogenous leukaemia. Clin Haematol 9: 55-86.

4. Heisterkamp N, Groffen J, Stephenson JR, Spurr NK, Goodfellow PN, et al. (1982) Chromosomal localization of human cellular homologues of two viral oncogenes. Nature 299: 747-749.

5. de Klein A, van Kessel AG, Grosveld G, Bartram CR, Hagemeijer A, et al. (1982) A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. Nature 300: 765-767.

6. Bartram CR, de Klein A, Hagemeijer A, van Agthoven T, Geurts van Kessel A, et al. (1983) Translocation of c-ab1 oncogene correlates with the presence of a Philadelphia chromosome in chronic myelocytic leukaemia. Nature 306: 277-280.

7. Groffen J, Stephenson JR, Heisterkamp N, de Klein A, Bartram CR, et al. (1984) Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. Cell 36: 93-99.

8. Lugo TG, Pendergast AM, Muller AJ, Witte ON (1990) Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. Science 247: 1079-1082.

9. Kelliher MA, McLaughlin J, Witte ON, Rosenberg N (1990) Induction of a chronic myelogenous leukemia-like syndrome in mice with v-abl and BCR/ABL. Proc Natl Acad Sci U S A 87: 6649-6653.

10. Daley GQ, Van Etten RA, Baltimore D (1990) Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. Science 247: 824-830.

11. Shepherd PC, Ganesan TS, Galton DA (1987) Haematological classification of the chronic myeloid leukaemias. Baillieres Clin Haematol 1: 887-906.

12. Deininger M, Buchdunger E, Druker BJ (2005) The development of imatinib as a therapeutic agent for chronic myeloid leukemia. Blood 105: 2640-2653.

13. Michiels JJ, Prins ME, Hagermeijer A, Brederoo P, van der Meulen J, et al. (1987) Philadelphia chromosome-positive thrombocythemia and megakaryoblast leukemia. Am J Clin Pathol 88: 645-652.

14. Michiels JJ, Berneman Z, SchroyensW (2004) Philadelphia chromosome positieve thrombocythemia without features of chronic myeloid leukemia in peripheral blood: natural history and diagnostic differentiation from Ph-negative essential thrombocythemia. Ann Hematol 83: 504-512.

15. Georgii A, Vykoupil KF, Thiele J (1980) Chronic megakaryocytic granulocytic myelosis-CMGM. A subtype of chronic myeloid leukemia. Virchows Arch A Pathol Anat Histol 389: 253-268.

16. Georgii A, Vykoupil KF, Buhr T, Choritz H, Döhler U, et al. (1990) Chronic myeloproliferative disorders in bone marrow biopsies. Pathol Res Pract 186: 3-27.

17. Georgii A, Buhr T, Buesche G, Kreft A, Choritz H (1996) Classification and staging of Ph-negative myeloproliferative disorders by histopathology from bone marrow biopsies. Leuk Lymphoma 22 Suppl 1: 15-29.

18. Georgii A, Buesche G, Kreft A (1998) The histopathology of chronic myeloproliferative diseases. Baillieres Clin Haematol 11: 721-749.

19. Thiele J, Schneider G, Hoeppner B, Wienhold S, Zankovich R, et al. (1988) Histomorphometry of bone marrow biopsies in chronic myeloproliferative disorders with associated thrombocytosis--features of significance for the diagnosis of primary (essential) thrombocythaemia. Virchows Arch A Pathol Anat Histopathol 413: 407-417.

20. Thiele J, Zankovich R, Schneider G, Kremer B, Fischer R, et al. (1988) Primary (essential) thrombocythemia versus polycythemia rubra vera. A histomorphometric analysis of bone marrow features in trephine biopsies. Analyt Quat Cytol Histol 10: 375.

21. Thiele J, Kvasnicka HM, Werden C, Zankonich R, Diehl V, et al. (1996). Idiopathic primary osteo-myelofibrosis: A clinicopathological study of 208 patients with special emphasis on evolution of disease features, differentiation from essential thrombocythemia and variables of prognostic impact. Leuk Lymphoma 22: 303-317.

22. Thiele J, Kvasnicka HM, Deihl V, Fischer R, Michiels JJ (1999) Clinicopathological diagnosis and differential criteria of thrombocythemia in various myeloproliferative disorders by histopathology, histochemistry and immunostaining from the bone marrow. LeukemiaLymphoma 33: 207-218.

23. Michiels JJ, Thiele J (2002) Clinical and pathological criteria for the diagnosis of essential thrombocythemia, polycythemia vera, and idiopathic myelofibrosis (agnogenic myeloid metaplasia). Int J Hematol 76: 133-145.

24. Michiels JJ, Kvasnicka HM, Thiele J (2005) Myeloproliferative Disorders:current perspective on diagnostic criteria, histopathology and treatment in essential thrombocythemia, polycythemia vera and chronic idiopathic myelofibrosis. Much: Verlag ME-Uwe Grunwal 1-106.

25. Michiels JJ, De Raeve H, Berneman Z,, Van Bockstaele D,, Hebeda K, et al. (2006) The 2001 World Health Organization (WHO) and updated European clinical and pathological (ECP) criteria for the diagnosis, classification and staging of the Ph1-chromosome negative chronic myeloproliferative disorders (MPD). Sem Thromb Hemostas 32: 307-340.

26. Michiels JJ, Kutti J, Stark P, Bazzan M, Gugliotta L, et al. (1999) Diagnosis, pathogenesis and treatment of the myeloproliferative disorders essential thrombocythemia, polycythemia vera and essential megakaryocytic granulocytic metaplasia and myelofibrosis. Neth J Med 54: 46-62.

27. Kvasnicka HM, Thiele J, Schmitt-Graeff (2001) Prognostic impact of bone marrow erythropoietic precursors cells and myelofibrosis at

diagnosis of Ph+ chronic myelogenous leukemia: a multicenter study on 495 patients. Brit J Haematol 113: 727-739.

28. Mason JE Jr, DeVita VT, Canellos GP (1974) Thrombocytosis in chronic granulocytic leukemia: incidence and clinical significance. Blood 44: 483-487.

29. Michiels JJ. Erythromelalgia and Thrombocythemia. Thesis 1981, Erasmus University

30. Michiels JJ (1997) Erythromelalgia and thrombocythemia: a disease of platelet prostaglandin metabolism--thesis, Rotterdam, 1981. Semin Thromb Hemost 23: 335-338.

31. Michiels JJ, Berneman Z, Schroyens W, Koudstaal PJ, Lindemans J, et al. (2006) Platelet-mediated erythromelalgic, cerebral, ocular, and coronary microvascular ischemic and thrombotic manifestations in patients with essential thrombocythemia and polycythemia vera: a distinct aspirin-responsive andcoumadin-resistant arterial thrombophilia. Platelets 17: 528-544.

32. Michiels JJ, Berneman Z, Schroyens W, Koudstaal PJ, Lindemans J, et al. (2006) Platelet-mediated thrombotic complications in patients with ET: Reversal by aspirin, platelet reduction, and not by coumadin. Blood Cells Mol Dis 36: 199-205.

33. James C, Ugo V, Le Couedica, Vainchenker W (2005) A unique clonal JAK2 mutation leading to constitutive signaling causes polycythemia vera. Nature 534: 1144-1148.

34. Michiels JJ, Ten Kate FWJ, Koudstaal PJ, Van Genderen PJJ (2013). Aspirin responsive platelet thrombophilia in essential thrombocythemia and polycythemia vera. World J Hematol 2: 3042.

35. Tefferi A, Thiele J. Orazi A (2007) Proposals and rationale for revision of the World Health Organization diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis: recommendations from an ad hoc international expert panel. Blood 110: 1092-1097.

36. Tefferi A, Vardiman JW (2008) Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organization criteria and point-of-care diagnostic algorithms. Leukemia 22: 14-22.

37. Michiels JJ, De Raeve H, Hebeda K, Lam KH, Berneman Z, et al. (2007) WHO bone marrow features and European clinical, molecular, and pathological (ECMP) criteria for the diagnosis of myeloproliferative disorders. Leuk Res 31: 1031-1038.

38. Tefferi A, Vainchenker W (2011) Myeloproliferative neoplasms: molecular pathophysiology, essential clinical understanding, and treatment strategies. J Clin Oncol 29: 573-582.

39. Michiels JJ, Berneman Z, Schroyens W,LamKH, De Raeve H (2013). PVSG and the WHO versus European Clinical Molecular and Pathological (WHO-ECMP) criteria for prefibrotic myeloproliferative neoplasms. World J Hematol 2: 71-80.

40. Michiels JJ, Berneman Z, Schroyens W, De Raeve H(2015) Changing concepts on the diagnostic criteria of myeloproliferative disorders and the molecular etiology and classification of myeloproliferative neoplasms. From Dameshek 1950 to Vainchenker 2005 and beyond. Acta Haematol 133: 36-51.