REVIEW ARTICLE

Myeloproliferative syndromes: diagnosis and therapeutic options*

Andrzej Hellmann

Department of Hematology and Transplantology, Medical University, Gdańsk, Poland

KEY WORDS

chronic myeloproliferative syndromes, molecular abnormalities, targeted therapy

ABSTRACT

Myeloproliferative syndromes (MPS) are clonal proliferation of hematopoietic progenitor cells characterized by proliferation of 1 or a few cell lines such as granulocytic, erythroid, megakaryocytic or mastocytic. These syndromes include: chronic myeloid leukemia, polycythemia vera, essential thrombocythemia, myelofibrosis, chronic eosinophilic leukemia/hypereosinophilic syndrome, chronic neutrophilic leukemia and systemic mastocytosis. Diagnosis of MPS is often difficult due to need of differential diagnosis with reactive proliferation caused by primarily non-hematological factors. Differentiation of individual MPS forms is also difficult because of overlapping of particular clinical or laboratory adnormalities. Discovery of specific molecular aberrations in the last few years facilitates diagnostic procedures. The discovered gene mutations or their fusions are associated with production of proteins possessing tyrosine kinase properties. These discoveries resulted in the successful introduction of the targeted therapy with tyrosine kinase inhibitors in the recent years.

In 1951 Dameshek presented the concept of chronic myeloproliferative syndromes (MPS) including chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (MF).[1] This term was limited to forms of hematopoietic disorders, in which maturation of cells is preserved, and their natural course as opposed to acute leukemias is chronic (few to several years). Dameshek's concept assumed that these syndromes result from the disturbances of mechanisms regulating normal hematopoiesis. The discovery of the Philadelphia (Ph) chromosome made in 1960, consisting of constant cytogenetic aberration in CML cells, the G6PD isoenzyme phenomenon analysis in heterozygotic women started by Fiałkow in the 70s of the last century, and clonogenic assays introduced simultaneously by Bradley and Metcalf, confirmed that the carcinogenic mutation of multipotential stem cells lies behind MPS.[2] The World Health Organization (WHO) classification implemented in 2001, alongside the classic forms proposed by Dameshek, also included hypereosinophilic syndrome/chronic eosinophilic leukemia (CEL) and chronic neutrophilic leukemia (CNL) to the group of MPS.[3] In the last few years the list of chronic MPS has been completed by adding systemic mastocytosis (SM).[4]

The incidence of chronic MPS is estimated at 6–9 new cases per 100 thousand annually. New cases occur most commonly between 40 and 60 years of age, occasionally under the age of 20 years. Chronic myeloproliferative diseases in the initial phase are characterized by a mild course with effective hematopoiesis and overproduction of a particular cell line and with more or less pronounced tendency to bone marrow metaplasia in the spleen, which leads to splenomegaly. Ineffective hematopoiesis and the appearance of blast forms in bone marrow and peripheral blood are observed after a few or several years, and are usually synonymous with transformation to acute myeloid leukemia. Because of advancements in molecular biology, molecular indices of the majority of these diseases have been determined in the recent years. In 1984 the *BCR/ABL* fusion gene, the result of translocation between the 9 and 22 chromosome (Ph chromosome) was described.[5] In 1993 *KIT* gene mutation characteristic of SM,[6] in 2003 *FIP1L1/PDGFRA* fusion gene, a PBE marker,[7] and in 2005 *JAK-2* gene mutation, found in the majority of PV cases in at least half of ET and MF cases and also in about 20% of PBN cases were characterized.[8]

All hither to described gene mutations cause overproduction of proteins of tyrosine kinase

Correspondence to:
Andrzej Hellmann, MD, PhD, Katedra i Klinika Hematologii i Transplantologii, Akademia Medyczna, ul. Dębinki 7, 80-952 Gdańsk, Poland, phone: +48-58-349-22-30, fax: +48-58-349-22-33, e-mail: klhem@amg.gda.pl
Received: April 17, 2008.
Accepted: April 25, 2008.
Conflict of interest: none declared.
Pol Arch Med Wewn. 2008; 118 (12): 756-760
Translated by Professional Language Services Sigillum Sp. z o.o., Kraków

* This article is based on the lecture which was presented at the 36th Congress of the Polish Society of Internal Medicine, Warszawa, Poland, April 25, 2008

properties, responsible for proliferation of particular cell lines. Thus, in the majority of these diseases a molecular marker facilitating diagnostics has been described. The proposed new WHO criteria, issued in August 2008, use molecular criteria to a large measure.[9] According to new WHO classification proposals, based on the assumption that molecular aberrations are an irrefutable argument that hematopoiesis in these syndromes is oncogenic, it is postulated to substitute the term "myeloproliferative syndromes", proposed by Dameshek, with "myeloproliferative cancers". Proposed changes in diagnostic algorithms concern most of all PV, ET and MF, and stress the significance of *JAK2* gene mutation analysis and histological evaluation of the bone marrow biopsy. Besides the diagnostic utility, known molecular aberrations, resulting in translation of proteins possessing properties of tyrosine kinases, provided a breakthrough in the therapy of these diseases. Known tyrosine kinases became a target for rapidly developing targeted therapy. From the beginning of the 21st century a new drug, imatinib, effective in CML and CEL therapy, is available. Second generation inhibitors of tyrosine kinases, including dasatanib and nilotinib, have been introduced in 2007. Other drugs are being evaluated in clinical trials. These drugs inhibit BCR/ABL, PDGFR and KIT tyrosine kinases.

Chronic myeloid leukemia The incidence of CML is 1–1.5 new cases per 100,000 people annually. The majority of cases are diagnosed on the basis of the routine blood cell counts in asymptomatic patients. The remaining patients present to the physician with symptoms resulting from splenomegaly. White blood cell counts at the diagnosis persist usually in the range of 30–100 G/l. The diagnosis has to be confirmed by the presence of Ph chromosome or BCR/ABL transcript using **poly**merase chain reaction (PCR) technique. In the case of high leukocytosis the cytoreductive treatment with hydroxyurea is started. If leukocytosis <50 G/l is observed, the treatment with BCR/ABL tyrosine kinase inhibitor – imatinib in a dose of 400 mg/d (1 tablet in the morning) is initiated. Treatment should lead to complete cytogenetic remission (observed in >80% of patients). The molecular response is also awaited. In the case of primary or secondary resistance a drug dose can be increased to 600–800 mg or switched to second generation tyrosine kinase inhibitors – dasatanib or nilotinib. Such patients should be monitored with cytogenetic tests and PCR. In younger patients who have a compatible bone marrow donor allogeneic bone marrow transplantation should be considered.[11,12]

Polycythemia vera PV is diagnosed in asymptomatic patients during the routine blood cell count analysis or, more commonly, on the basis of skin and mucous membrane redness or splenomegaly. Symptoms suggestive of polycythemia involve headaches and drowsiness, vision disturbances, erythromelalgia, pruritus after contact with water and traits of gout. The most severe complications of polycythemia are thrombotic and hemorrhagic by nature as well as hypertension. The proposal of new revised diagnostic criteria significantly simplifies the diagnosis of PV.[9] Accordingly, 2 major diagnostic criteria must be fulfilled: hemoglobin level >18.5 g/dl for men or 16.5 g/dl for women and *JAK-2* gene mutation (V261F or other functionally similar), and 1 out of minor criteria, among which serum erythropoietin level within the reference range is crucial. When the *JAK-2* gene mutation is absent 2 remaining minor criteria must be fulfilled, that is histological bone marrow biopsy assessment presenting high cellularity with marked erythroid, granulocytic and megakaryocytic line proliferation, and the demonstration of the erythroid colonies increase without the addition of erythropoietin to the culture in clonogenic tests. In low-risk patients (<60 years old, with no history of thrombotic events) and with normal white blood cell and platelet counts, use of low-dose acetylsalicylic acid and repeated phlebotomy are sufficient. The cytoreductive treatment is recommended in patients aged >60 years or with a history of thrombotic events and in patients with leukocytosis >15 G/l or platelet count >1000 G/l or with massive splenomegaly. Hydroxycarbamide (1000–2000 mg/d) is most often used. Interferon α is an alternative in younger patients (3 million units 3 times a week).[11,13]

Essential thrombocythemia In over 50% of patients the onset of ET is asymptomatic and the disease is diagnosed on the basis of blood cell count analysis and increased platelet count. In some patients symptoms resulting from thrombosis or bleedings are observed. Erythromelalgia is relatively commonly noted. In diagnostic management first of all secondary causes of thrombocytosis, that is infections, inflammatory diseases, sideropenias and neoplastic diseases should be excluded. The proposed new diagnostic criteria for ET decrease the required platelet count from 600 to 450 G/l.[9] Such a change enables early diagnosis of the disease. The next criterion of diagnostic importance is *JAK-2* gene mutation (*V617F*), observed in about 50% of patients. Until very recently it has been believed that mutation of this gene may be observed at codon 617 only; however, the recent publications indicate the possibility of different gene mutations, especially at exon 12. Moreover, in some of the ET cases (5-10%) *MPL* gene mutation is observed (W515L). The third important criterion is histological bone marrow evaluation, presenting megakaryocytic proliferations without proliferation of erythroid and granulocytic lines. When the *JAK-2* mutation is absent, exclusion of the *BCR/ABL* fusion gene is important. The WHO criteria do not consider CRP level, which if remains within the reference range releases the diagnostician from the duty of seeking causes of secondary thrombocythosis.

If *JAK-2* gene mutation is diagnosed, the hemoglobin level with iron and ferritine levels should be analyzed in order to exclude PV. Myelofibrosis is excluded on the basis of histological evaluation of the bone marrow without fibrosis. Cytoreductive treatment is indicated in high-risk patients. Each of the following criteria allows use of hydroxyurea (usually in a dose of 1000–1500 mg/d): age >60 years, occurrence of thrombotic or hemorrhagic incidents in a history and platelet count >1500 G/l or >1000 G/l in case of risk of cardiovascular complications. In younger patients and in those who do not response to hydroxyurea, anagrelid in a dose of 1.5–3.0 mg/d or interferon α should be considered.[11,14]

Myelofibrosis In about 30% of patients with myelofibrosis the diagnosis is made on the basis of the results of blood cell counts without determined symptoms. In over half of patients symptoms of splenomegaly make MF diagnosis easier; in the remaining patients the following diseases influence the clinical presentation: anemia, splenomegaly and traits of neutropenia or thrombocytopenia associated with progressing bone marrow fibrosis or features of extramedullary metaplasia in various organs. According to the current WHO proposals the histological bone marrow biopsy revealing proliferation and atypia of megakaryocytes, accompanied by fibrosis is the key diagnostic criterion.[9] The second criterion is *JAK-2* or *MPL* gene mutation, and in case of its absence – exclusion of *BCR/ABL* mutation. Definitive MF diagnosis requires the fulfillment of at least 2 from among 4 minor criteria: the leukoerythroblastic reaction in peripheral blood, increased lactate dehydrogenase level, anemia and palpable spleen on physical examination. Age >60 years, hemoglobin level <10 g/dl, platelet count <100 G/l and >3% blast cells in the peripheral blood sample are recognized as a poor prognostic factor. In this situation allogeneic hematopoietic cell transplantation considering the so-called reduced conditioning is the strongest recommended management. The latest reports indicate about 58% survival within 3 years following such management and the transplantation-related mortality about 32%. In the last few years MF therapy has been attempted with thalidomide. The response to such treatment is observed in the form of improvement in parameters of red blood cells and platelets and a decrease in spleen size. In the proliferation phase of MF use of hydroxyurea is recommended. Reports on the effect of the action of new molecules inhibiting the mutated *JAK-2* gene have been published recently. Clinical trials involve most of all patients with MF with *JAK-2* mutation. Results of these trials are still underway. It is too early to discuss the effectiveness of these drugs and possible side or undesirable effects, in particular. In the center of other important signalling pathways is *JAK-2* kinase. Moreover, these drugs may also influence the non-mutated *JAK-2* gene product.[11,15]

Chronic eosinophilic leukemia Only in about 10% of patients diagnosis is made accidentally on the basis of blood morphology analysis, the remaining patients usually come to a physician with symptoms of fatigue, fever, cough, muscular pain and diarrhoea. Eosinophilic infiltrations in the heart muscle, skin, lungs and the nervous system cause specific clinical symptoms. Persistent hypereosinophilia in peripheral blood >1.5 G/l is fundamental for diagnosis. All possible causes of reactive hypereosinophilia, secondary to allergic diseases, parasitic diseases, connective tissue diseases (collagen diseases), neoplastic diseases (especially T-cell lymphomas), Hodgkin's lymphoma and other myeloproliferative diseases, in which eosinophilic cells are part of the carcinogenic clone (CML, acutemyeloid type M4 with eosirophilia leukemia, PV, NS, MF) should be excluded to make a diagnosis. The most important criterion confirming diagnosis of CEL leukemia is detection of the *FIP1L1/PDGFRA* fusion gene. In this situation imatinib in a dose of 100 mg/d is the therapy of choice (with excellent therapeutic effect). CEL can also be diagnosed in patients with hypereosinophilia when blast cells in peripheral blood (≥2%) or in the bone marrow (≥5%) are observed; however, blast count cannot exceed 20%. In the remaining cases, once all causes of reactive eosinophilia are excluded, idiopathic hypereosinophilia is diagnosed. Now the use of the term hypereosinophilic syndrome is not recommended. Treatment begins with corticosteroids administration, and when no therapeutic response is obtained cytoreductive treatment (hydroxycarbamide in a dose of 1.5–2 g) is used.[16,17]

Chronic neutrophilic leukemia CNL is a very rare myeloproliferative disease, characterized by persistent neutrophilia in peripheral blood and splenomegaly. The Ph chromosome and the *BCR/ABL* fusion gene are not observed. Diagnosis is made by exclusion of other causes of neutrophilic leukocytosis. In 20% of patients *JAK-2* gene mutation have been discovered.

Systemic mastocytosis The hematological picture rarely indicates this diagnosis, because the majority of mast cells mature outside the bone marrow – in the liver, spleen, lymphatic nodes and perivascular tissue. The majority of symptoms result from the release of mediators (histamine). They include abdominal pains, bronchospasm, headaches and erythema. Skin symptoms, e.g. urticaria and pruritus, and bone symptoms, like bone pain, arthralgias and bone fractures are also observed. Physical examination shows splenomegaly . The main diagnostic criterion is accumulation and aggregates of mastocytes (>15 cells) in the bone marrow. Minor criteria are: atypia of at least 85% of mast cells and found in 80% of patients *KIT* gene mutation at codon 816 (D816V), rarely at other codons, e.g. 522 (F522C). The remaining minor criteria are coexpression of CD117, CD2 and CD25 antigens on

mastocytes and increased serum tryptase levels (>20 ng/ml). Diagnosis can be confirmed if a patient fulfils the major criterion and 1 out of 4 minor criteria, or 3 out of 4 minor criteria.[4] Antihistamines are used in symptomatic therapy and in the prophylaxis. Dasatanib appeared to be an effective drug in patients with *KIT D 816V* gene mutation. Imatinib is the drug of choice in other *KIT* gene mutations (i.e. F522C). Cladribine, hydroxycarbamide or α-interferon are used in the absence of the mutation.[18,19]

In about 10–15% of patients suspected of MPS it is not feasible to make a specific diagnosis on the basis of the present criteria. This is usually observed in the initial phase of one of the described here forms of MPS. At that time molecular tests will be helpful, but when *JAK-2* gene mutation is present, differentiation between PV, ET or MF might still be a challenge. Further follow-up could be of help. The other cause of diagnostic problems can be establishment of the diagnosis in the late stage of the disease, in which there is bone marrow fibrosis or acceleration or blastic phase.

REFERENCES

1 Dameshek W. Some speculations on myeloproliferative syndromes. Blood. 1951; 6: 372-375.

2 Adamson JW. The pathogenesis of myeloproliferative syndromes. Br J Haemat. 1978; 38: 299-303.

3 Vardiman JW, Harris NL, Brunning RD. WHO classification of the myeloid neoplasms. Blood. 2002; 100: 2292-2302.

4 Valent P, Horny HP, Esribano L, et. al. Diagnostic criteria and classification of mastocytosis. Leuk Res. 2001; 25: 603-625.

5 Groffen J, Stephenson JR, Heisterkamp N, et al. Philadelphia chromosomal breakpoints are clustered within a limited region bcr on chromosome 22. Cell. 1984; 36: 93-96.

6 Furitsu T, Tsujimura T, Tono T, et. al. Identifications of mutations in the coding sequence of proto-oncogene c-Kit in a human mast cell leukemia. J Clin Invest. 1993; 92: 1736-1744.

7 Cools J, De Angelo DJ, Gotlieb J, et al. A tyrosine kinase created by fusion of PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N Engl J Med. 2003; 348: 1201-1214.

8 Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK-2 in myeloproliferative disorders. N Engl J Med. 2005; 352: 1779-1790.

9 WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues ed. ed. S.H. Swerdlow, E. Campo, N.L. Harris, et al. JARC, Lyon 2008.

10 Hellmann A. Targeted therapy in proliferative diseases of haematopoietic system. Acta Haemat Pol. 2006; 37 (Suppl. 1): 35-39.

11 Hellmann A, Bieniaszewska M, Prejzner W. Myeloproliferative syndromes. In: Recommendations for diagnostic and therapeutic management of cancers. Onkol Prakt Klin. 2007; 3 (Suppl. C): 476-487.

12 Hellmann A, Hołowiecki J, Jędrzejczak WW, et al. Aktualne uzgodnienia dotyczące stosowania dazatynibu u chorych na przewlekłą białaczkę szpikową lub ostrą białaczkę limfoblastyczną z obecnością chromosomu Ph. Acta Haemat Pol. 2007; 38: 123-138.

13 Landolfi R, Di Gennaro L, Barbui T. Leukocytosis as a major thrombotic risk factor in patients with polycythemia vera. Blood. 2007; 109: 2446-2452.

14 Carobbio A, Finazzi G, Guerini V, et al. Leucocytosis is a risk factor for thrombosis in essential thrombocythemia: interaction with treatment, standard risk factors and JAK-2 mutation status. Blood. 2007; 109: 2310-2313.

15 Barosi G, Besses C, Birgegard G, et al. A unified definitions of clinical resistance/intolerance to hydroxyurea in essential thrombocythemia: results of a consensus process by international working group. Leukemia. 2007; 21: 277-280.

16 Prejzner W, Szatkowski D, Wassąg B, et al. Celowana terapia imatinibem u chorego z przewlekłą białaczką eozynofilową. Wsp Onkol. 2005; 9: 7-10.

17 Baccarani M, Cilloni D, Rondoni M, et al. The efficacy of imatinib mesylate in patients with FIP1L1 PDGFRA positive hypereosinophilic syndrome. Haematologica. 2007; 92: 1173-1179.

18 Valent P, Akin C, Sperr WR. Agressive systematic mastocytosis and related mast cell disorders current treatment options and proposed response criteria. Leuk Res. 2003; 27: 635-641.

19 Garcia-Montero AC, Jara-Acevedo M, Teodosio C, et al. KIT mutations in mast cell and other bone marrow hematopoietic cell lineages in systemic mast cell disorders. Blood. 2006; 108: 2366-2372.