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Recent advances in pathogenesis and treatment of classical Philadelphia-negative myeloproliferative neoplasms

Aktualne postępy w patogenezie i leczeniu klasycznych nowotworów mieloproliferacyjnych Filadelfia-ujemnych

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Summary

In the last few years we have witnessed a great progress in our understanding of the pathogenesis of myeloproliferative neoplasms (MPNs). The classical Philadelphia-negative MPNs include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). They were grouped together based on similar clinical phenotype by Dameshek in 1951. Since then the identification of JAK2V617F and MPL mutations has changed our view on disease pathogenesis and led to the revision of World Health Organization (WHO) diagnostic criteria for classical Philadelphia-negative MPNs. A new classification issued in 2008 incorporated these molecular abnormalities and therefore has modified the diagnostic approach. Moreover, these mutations have modified the strategies for monitoring and response assessment and they could become the potential targets for small-molecule agents. In this review we present current overview on molecular basis of MPNs including novel mutations and discuss the diagnostic criteria and therapeutic modalities.

Key words: myeloproliferative neoplasms, polycythemia vera, essential thrombocythemia, primary myelofibrosis, JAK2V617F

Streszczenie

W ostatnich kilku latach jesteśmy świadkami ogromnego postępu jaki dokonał się w zrozumieniu patogenezy nowotworów mieloproliferacyjnych (MPNs). Klasyczne nowotwory mieloproliferacyjne Filadelfia-ujemne obejmują nadkrwistość prawdziwą, nadpłytkowość samoistną i pierwotne włóknienie szpiku. Wszystkie te jednostki chorobowe wykazują podobieństwo obrazu klinicznego i zostały zgrupowane razem przez Dameshka w roku 1951. Od tego czasu, w związku z odkryciem mutacji JAK2V617F i MPL zmieniło się nasze spojrzenie na patogenezę tych chorób i doprowadziło do rewizji dotychczasowych kryteriów diagnostycznych Światowej Organizacji Zdrowia (WHO). Nowa klasyfikacja WHO została zaproponowana w roku 2008 i objęła ona niedawno opisane nieprawidłowości molekularne, zmieniając zasadniczo aktualne podejście diagnostyczne. Co więcej, mutacje te zmodyfikowały sposób monitorowania chorób, oceny efektów leczenia, a także stały się potencjalnym terapeutycznym celem dla nowych cząsteczek. W pracy przedstawiono aktualne spojrzenie na molekularną patogenezę MPNs, z podkreśleniem znaczenia nowych mutacji genetycznych. Poddano dyskusji kryteria diagnostyczne i dostępne opcje lecznicze.

Słowa kluczowe: nowotwory mieloproliferacyjne, nadkrwistość prawdziwa, nadpłytkowość samoistna, pierwotne włóknienie szpiku, JAK2V617F

INTRODUCTION

The myeloproliferative neoplasms (MPNs) comprise a group of clonal hematopoietic stem cell disorders characterized by proliferation of one or more myeloid lineage. MPNs occur mainly in adults between 5th and 7th decade of life, and the annual incidence is estimated to be 6-10 per 100 000 population (1). The term "myeloproliferative disorders" (MPDs) was proposed in 1951 by Dameshek to encompass several disorders with similar clinical phenotype. They included polycythemia

vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), chronic myelogenous leukemia (CML) and erythroleukemia (2). The last 5 years have brought a pivotal progress in our understanding of the pathogenesis of classical Ph-negative MPDs. It was associated with the identification of the specific molecular marker- the JAK2V617F point mutation, which seems to be involved in PV, ET and PMF (3). It is now clear that patients with this mutation are biologically distinct from those without the mutation and that the muta-

tion is associated with different disease phenotype. Moreover, several novel mutations have been recently identified, but their pathogenic and prognostic relevance has not been established yet (4). The current World Health Organization (WHO) classification for hematological malignancies has incorporated the recently discovered mutations into the diagnostic approach. The term "MPDs" has been changed to "MPNs". The WHO classification includes 8 clinical entities and it was presented in table 1 (5).

Table 1. The World Health Organization Classification of Myeloid Malignancies (5).

Myeloproliferative neoplasms (MPNs)
I Classical MPNs
Chronic myelogenous leukemia, BCR-ABL1 positive Polycythemia vera Essential thrombocythemia Primary myelofibrosis
II Non-classical MPNs
Chronic neutrophilic leukemia Chronic eosinophilic leukemia, not otherwise specified Mastocytosis Myeloproliferative neoplasms, unclassifiable

This current review will focus on classical Ph-negative MPNs and includes molecular basis of pathogenesis, risk stratification, diagnostic and therapeutic management.

MOLECULAR BASIS FOR MYELOPROLIFERATIVE NEOPLASMS

JAK2- mutated MPNs

JAK2 is located on chromosome 9p24 and it belongs to the Janus family of non-receptor protein tyrosine kinase. Janus kinase/signal transducer and activator of transcription (JAK-STAT) signaling remains crucial for survival and normal function of hematopoietic and other cells (6). The JAK2V617F mutation is of particular relevance to MPNs including mainly PV, ET and PMF (3). The JAK2V617F results from a somatic G to T mutation involving JAK2 exon 14. It leads to nucleotide shift at position 1849 and the substitution of valine to phenylalanine at codon 617. Mutated JAK2 causes cytokine-independent growth of cells and their hypersensitivity to cytokines (7). The JAK2V617F point mutation is found in approximately 95% of patients with PV and 40-50% of patients with ET and PMF (3, 8). Two-step model may explain the development of cells which are homozygous for JAK2 mutation. Initially, the occurrence of the JAK2V617F gives rise to a heterozygous clone which replaces normal hematopoietic cells. The second step includes mitotic recombination within cells heterozygous for the JAK2V617F with subsequent uniparental disomy. As a consequence, the daughter cells are homozygous for JAK2V617F and they replace the heterozygous clone. It may happen that myeloid cell population includes a

variable proportion of JAK2 mutant alleles; it means that cells with JAK2 mutation may coexist with cells lacking this abnormality. The first step is characterized by progressive increase of mutant alleles from 0 to 50%, whereas the second step from 50 to 100% (9). The experimental studies in mouse model demonstrated that both heterozygous and homozygous JAK2 mutations induce PV-like disease phenotype, but homozygous JAK2 mutation is associated with more aggressive disease with progression to post-PV myelofibrosis (10). The JAK2V617F homozygosity is frequently seen in PV and PMF. In contrast, high mutant allele burden is rarely seen in ET (11). Homozygosity in granulocytes can be detected in 30% of patients with PV (3), but it was demonstrated in approximately 90% of colonies of hematopoietic progenitor cells derived from patients with PV whereas homozygous progenitor colonies were not observed in ET (12). This difference in the occurrence of homozygosity may result from the substantial variation in the rate of mitotic recombination among persons and the role of genetic and environmental factors determining susceptibility to mitotic recombination is very likely (11). However, it should be clearly underlined that variability in the allele burden is not sufficient to distinguish different clinical entities. It does not help us to answer the question how one mutation gives rise to three different clinical phenotypes. Moreover, there is an increasing number of evidence that the JAK2V617F is not an initiating event in MPNs and that pre-JAK2 mutated clone may exist (13). Some studies suggest that deletions of 20q and other cytogenetic abnormalities are present in about 10% of patients with MPNs and that this deletion may occur before the JAK2 mutation. It was demonstrated that deletions 20q are exclusively V617F-positive (14). In conclusion, the pathogenic role of JAK2V617F mutation requires to be better defined, especially in the light of the presence of novel mutations which may occur in patients with MPNs (4).

As it was aforementioned, JAK2 allele burden is associated with clinical phenotype and PV patients homozygous for V617F mutation have been found to have a more severe disease (15). Recently published report has demonstrated that patients with an allele burden equal or > 50% had higher white blood cell (WBC) count, greater spleen size and lower platelet count than those with less than 50% of mutant dosage. It was also proved that patients homozygous for JAK2 mutation had significantly higher risk of developing myelofibrosis (16).

JAK2 exon 12 mutations were identified in PV who were negative for JAK2V617F point mutation. Those patients presented with an isolated erythrocytosis, distinct bone marrow morphology and low serum erythropoietin level. It is noteworthy that erythroid colonies have grown from their blood samples in the absence of exogenous erythropoietin. In contrast to JAK2V617F-positive PV patients, JAK2 exon 12 mutated PV subset is often heterozygous for this mutation (17).

MPL mutations

MPL (myeloproliferative leukemia virus oncogene) located on chromosome 1p34, is the thrombopoietin receptor. MPL is highly expressed in early hematopoietic progenitors and in cells of megakaryocytic lineage. MPL is the key growth and survival factor for megakaryocytes (18). The two most common MPL mutations are W515L and W515K. They were found in about 10% of patients with PMF and in less than 10% of ET-patients lacking the JAK2V617F mutation (19). MPL-mutated ET patients are older, have lower hemoglobin concentration, higher platelet count and higher risk of arterial thrombosis (20) whereas the presence of MPL mutation in patients with PMF is associated with female gender, older age, lower hemoglobin concentration and blood transfusion dependence (21).

TET2 mutations

TET2 is located on chromosome 4q24 and its function is probably associated with epigenetic regulation of transcription (22). TET2 mutations are present in about 10-15% of patients with MPNs, including both the JAK2V617F – positive and negative cases (23). It should be highlighted that TET2 mutations may coexist with other relevant mutation e.g. KITD816V for mast cell disease (24). Moreover, it was demonstrated that TET2 mutations may precede the acquisition of a JAK2 mutation. Up-to-date studies show that the presence of TET2 mutations in MPNs do not affect survival, leukemic transformation and the risk of thrombosis (23).

Novel mutations in MPNs

Several novel mutations have been recently found in patients with MPNs:

- 1) ASXL1 (Additional Sex Combs-Like 1,
- 2) CBL (Casitas B-lineage lymphoma proto-oncogene),
- 3) IDH1 and IDH2 (Isocitrate dehydrogenase 1 and 2),
- 4) IKZF1 (IKAROS family zinc finger 1),

- 5) LNK,
- 6) EZH2.

All these mutations are rarely seen in patients with chronic phase of MPNs whereas their frequency may exceed 20% in blast transformation (tab. 2) (4).

The results including the analysis of JAK2 haplotype may suggest that hereditary component confers susceptibility to MPNs and some of above-mentioned mutations may play a role at different stages of disease evolution. A strong association between the risk of developing a JAK2V617F-positive MPNs and a germline haplotype (46/1 or GGCC) has been recently demonstrated (fig. 1) (25).

2008 WHO DIAGNOSTIC CRITERIA FOR MPNS

General aspects

The discovery of JAK2 mutations (either V617F or JAK2 exon 12 mutations) in more than 95% of patients with PV was a turning point in the classification and diagnosis of all classical MPNs. There are several important issues. This genetic marker has been found only in myeloid neoplasms, but not in non-myeloid malignancies (8). Therefore, JAK2 mutation remains a sensitive marker of PV, but it is not specific for any one MPNs. The presence of JAK2 mutation may confirm the diagnosis of myeloid malignancy, but its absence does not exclude the MPNs. It should be underlined that this mutation is not seen in healthy volunteers (26). In PV and PMF patients the absence of JAK2 mutation has limited diagnostic utility since about half the patients lacking this marker (5). A small subset of patients with ET and PMF possess MPL mutations, but their incidence remains very low and therefore the diagnostic utility is highly limited (20). It is noteworthy, that there are lacking standardized methods of JAK2 assessment and therefore we should realize the possibility of false positive and negative results. It may occur when very sensitive diagnostic method was implemented or in case of low tumor burden e.g. mononuclear cells from peripheral blood were used for assay (27). In conclusion, the JAK2 and MPL mutations are not necessary to establish a diagnosis

Table 2. Novel mutations in classical myeloproliferative neoplasms (4,43) modified by authors.

Mutation	Chromosome location	Mutational frequency
JAK2 (Janus kinase 2) JAK2V617F exon 14	9p24	PV~96%; ET~55%; PMF~65%; BP~50%
JAK2 exon 12	9p24	PV~3%
MPL (myeloproliferative leukemia virus oncogene) exon 10	1p34	ET~3%, PMF~10%; BP~5%
TET2 (TET oncogene family member 2) exon 12	4q24	PV~16%; ET~5%; PMF~17%; BP~17%
ASXL1 (additional sex combs-like-1) exon 12	20q11.1	PMF~13%, BP~18%
CBL (casitas B-lineage lymphoma proto-oncogene) exon 8/9	11q23.3	PMF~6%
IDH1/IDH2 (isocitrate dehydrogenase) exon 4	2q33.3/15q26.1	PV~2%, ET~1%, PMF~4%, BP~20%
IKZF1 (IKAROS family zinc finger 1)	7p21	BP~19%
LNK exon 2	12q24.12	BP~10%
EZH2 (enhancer of zeste homolog 2) exons 10, 18 and 20	7q36.1	PV~3%, PMF~7%

Legend: PV = polycythemia vera, ET = essential thrombocythemia, PMF = primary myelofibrosis, BP = blast phase.

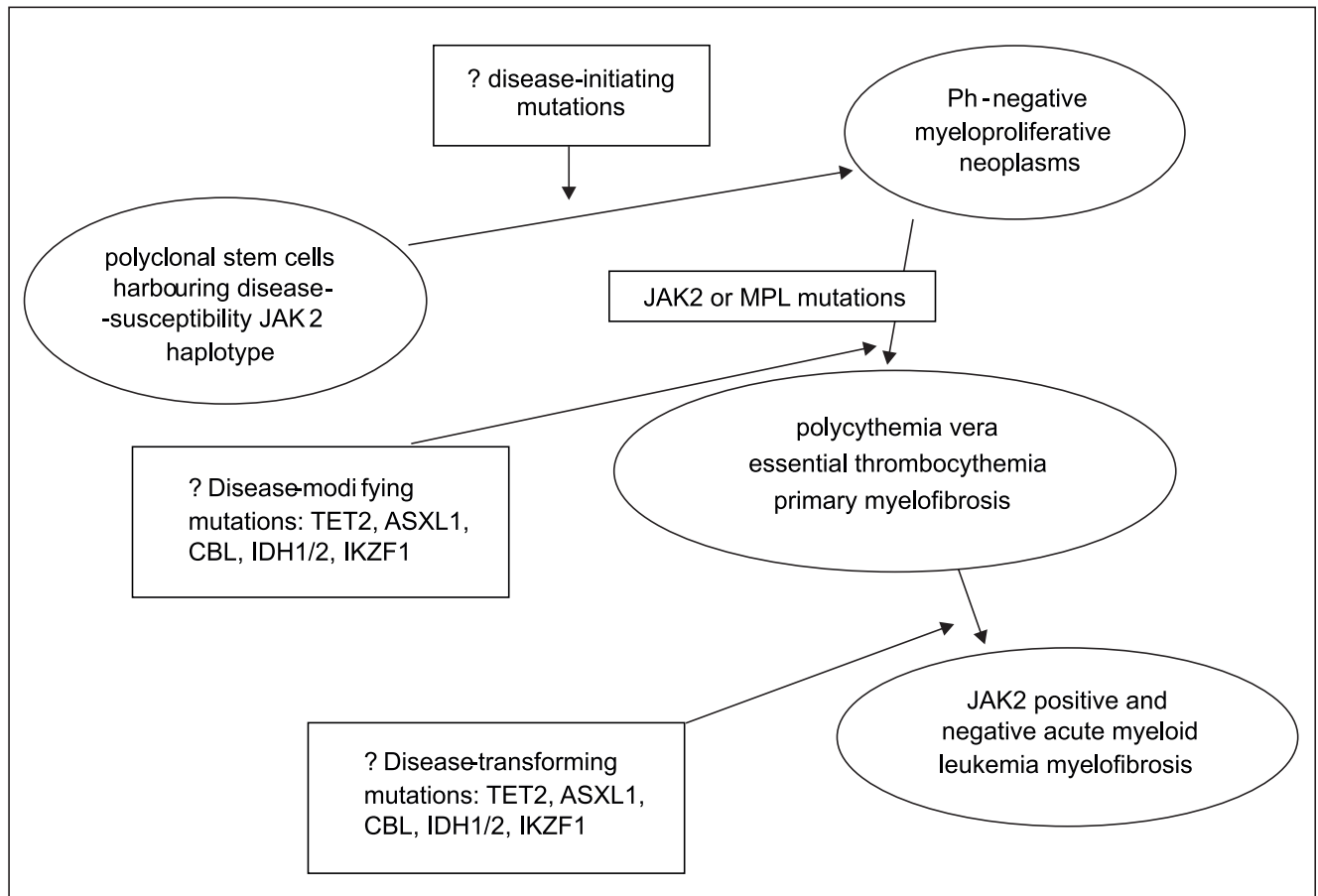


Fig. 1. Clonal evolution in myeloproliferative neoplasms (modified from 4).

of MPNs, but they could be helpful when present. If not, the diagnosis of MPNs is still likely. A diagnostic diagram using JAK2 genotyping was proposed in patients suspected to have MPNs (28) (fig. 2).

Due to above-mentioned ambiguities concerning JAK2 mutations, a new WHO classification system requires bone marrow histology which may help in distinguishing between 3 classical MPNs. Importantly, the 2008 WHO criteria include several additional, clinical and laboratory findings which are specific for particular MPNs.

Because of similarity with secondary causes, diagnosis of different MPNs remains challenging. The introduction of the new molecular markers seem to facilitate the final diagnosis, but the presence of mutations should be integrated with other characteristic findings.

POLYCYTHEMIA VERA (PV)

Definition: PV is a chronic myeloproliferative neoplasm characterized by increased red cell production. The presence of JAK2 mutations in virtually all patients with PV results in proliferation of not only erythroid, but also granulocytic and megakaryocytic lineages. We can distinguish three phases of disease: 1) pre-polycythemic phase with mild erythrocytosis 2) overt polycythemia with an increase of red cell mass and 3) myelofibrotic "spent" phase. PV may infrequently progress to acute myeloid leukemia or myelodysplastic syndrome (5).

Epidemiology: The annual incidence of PV varies between 0.7 to 2.6 per 100 000 population with a median age at diagnosis of 60 years and male predominance (5).

Clinical manifestations: The main clinical features encompass venous and arterial thrombosis. Most thrombotic events occur in the two years before diagnosis (29). Arterial thrombosis includes myocardial infarction, ischemic stroke and peripheral arterial occlusion. Venous thrombosis is represented by lower extremity deep venous thrombosis, pulmonary embolism and splanchnic vein thrombosis (28). The other symptoms include headache, visual disturbances, pruritus and erythromelalgia. There are hepatomegaly, splenomegaly and plethora on physical examination (30).

Diagnostic criteria: see table 3 (5).

Risk-stratification: Older age (> 60 years) and a previous history of thrombosis remain risk factors for thrombosis. In high-risk patients both factors are present whereas in low-risk neither of these is demonstrated. Intermediate risk group includes patients with cardiovascular risk factors, such as hypertension, smoking, hyperlipidemia and diabetes (28, 29). Moreover, recently published reports have showed that WBC count > 15.0 x 10⁹/L and high JAK2V617F allele burden are independent risk factor for thrombosis (15).

Treatment: The treatment should adhere the standard risk stratification. In low and intermediate-risk

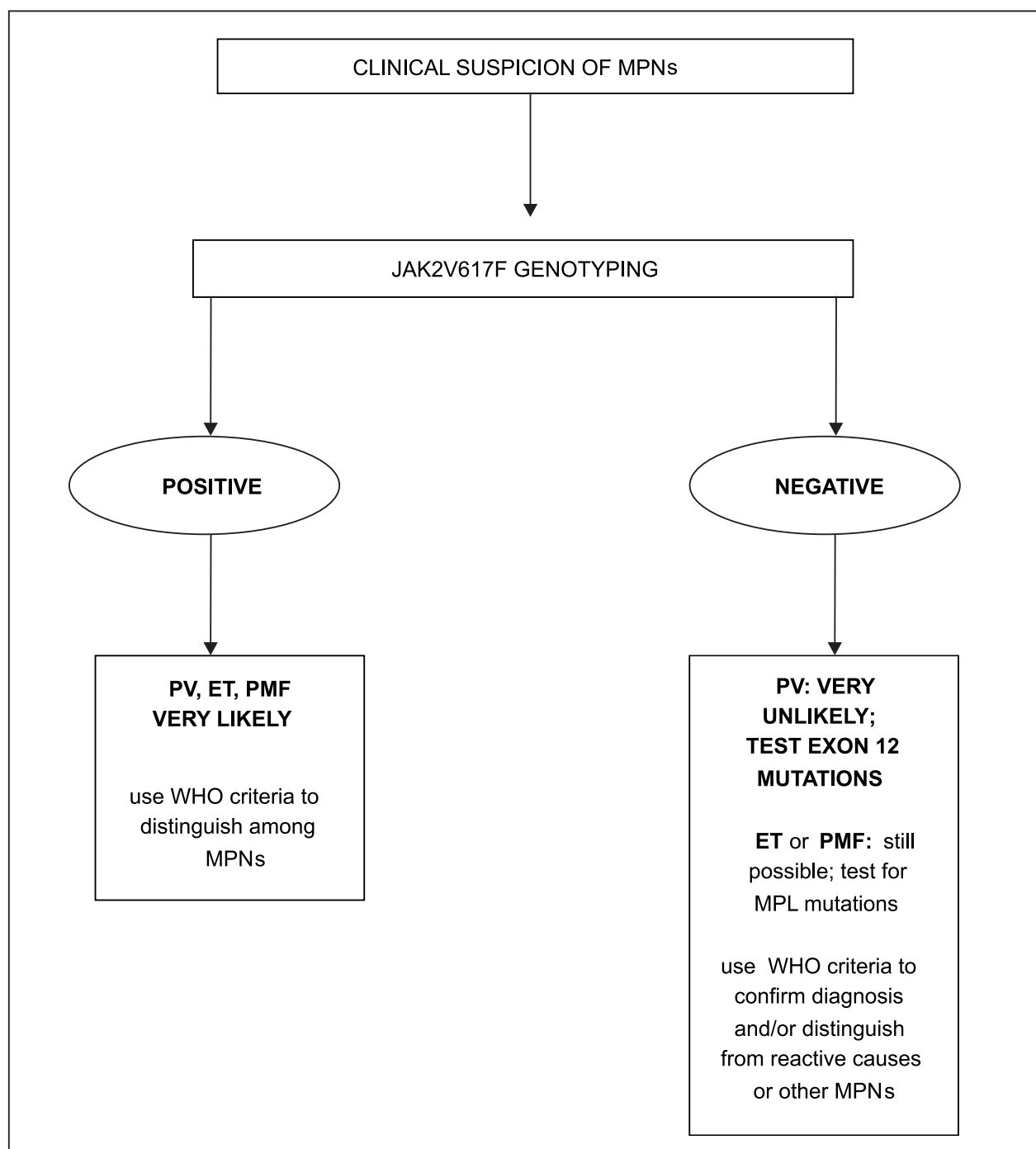


Fig. 2. The JAK2V617F genotyping in the diagnostic work-up in suspicion of MPNs (28).

Legend: ET = essential thrombocythemia, MPNs = myeloproliferative neoplasms, PMF = primary myelofibrosis, PV = polycythemia vera.

patients, phlebotomy and low dose of aspirin are recommended. High-risk patients require phlebotomy, low-dose aspirin and myelosuppressive agents (hydroxyurea or interferon-alpha). Either hydroxyurea or interferon-alpha is first-line cytoreductive therapy at any age (31).

Therapeutic management in pregnancy: Female-PV patients should be stratified according to risk factors which include 1) previous major thrombotic or bleeding complications and 2) previous pregnancy complications (pre-eclampsia, stillbirth, > 3 first-trimester or > 1 second and third-trimester losses). High-

risk pregnancy is defined by the presence of at least one of the above-mentioned risk factors. This subgroup should be treated with phlebotomy (target hematocrit below 45%), aspirin, low molecular weight heparin and interferon-alpha. In low-risk group (no risk factors present) phlebotomy, aspirin and low molecular weight heparin are recommended (31).

Prognosis: Mortality rate is age-dependent and it is estimated to be 1.6 fold and 3.3 fold higher than in the reference population in patients younger and older than 50 years respectively. Major causes of death are fatal thrombotic events and progression to AML (32).

Table 3. WHO Diagnostic Criteria for Polycythemia Vera (5).

Major criteria
1. Hgb > 18.5 g/dL (men) or 16.5 d/dL (women) or Hgb or Hct > 99 th percentile of reference range for age, sex, or altitude of residence or Hgb > 17 g/dL (men) or 15 g/dL (women) if associated with a documented and sustained increase of ≥ 2 g/dL from baseline that cannot be attributed to correction of iron deficiency or elevated red cell mass > 25% above mean normal predicted value
2. Presence of JAK2V617F or similar mutation
Minor Criteria
1. Bone marrow showing hypercellularity for age and trilineage growth
2. Subnormal serum Epo level
3. EEC growth
Diagnostic combinations
Both major criteria + 1 minor criterion or first major criterion + 2 minor criteria

Legend: EEC = endogenous erythroid colonies, Epo = erythropoietin, Hgb = hemoglobin, Hct = hematocrit.

ESSENTIAL THROMBOCYTHEMIA (ET)

Definition: ET is a chronic myeloproliferative neoplasm that involves the megakaryocyte lineage. ET is characterized by significant thrombocytosis in peripheral blood and increased numbers of mature megakaryocytes in the bone marrow (5).

Epidemiology: The annual incidence of ET is estimated to be 0.6-2.5 per 100 000 persons per year. Male and female are equally affected. A median age at diagnosis is 60 years, but it may occur in patients at the age of 30 years and infrequently in children (33).

Clinical manifestations: Thrombosis and hemorrhage represent main clinical symptoms. Some studies demonstrated that the cumulative rate of thrombosis during the disease course ranged from 1.9% to 3% per year (34). Involvement of the microcirculatory system is typical for ET and it may manifest as erythromelalgia, ischemic attacks, visual defects, headache and paresthesia (5). Bleeding resulting from acquired von Willebrand disease, occurs most commonly from mucosa of gastrointestinal and upper respiratory tracts (35).

Diagnostic criteria: see table 4 (5).

Risk stratification: Older age (> 60 years) and history of thrombosis are standard risk factors for ET (the same as it was mentioned for PV) (28, 29). Some reports suggested that patients with high WBC count had higher risk for thrombosis. The best leukocyte cutoff values for predicting the thrombotic events was found to be $9.4 \times 10^9/L$ (36). The British Study Group (MRC-PT1) presented risk stratification system for ET patients. High-risk group includes age > 60 years, prior thrombotic or bleeding events, vascular risk factors and platelet count > $1000 \times 10^9/L$, for intermediate group belong ET patients at the age between 40-60 years with no vascular risk factors and no history of thrombosis/bleeding and with a platelet count less than $1500 \times 10^9/L$. The low-risk patients fulfill the criteria for intermediate group but are younger than 40 years (37).

Table 4. WHO Diagnostic Criteria for Essential Thrombocythemia (5).

Major criteria
1. Sustained platelet count ≥ $450 \times 10^9/L$ Bone marrow showing proliferation mainly of the megakaryocytic lineage with increased numbers of enlarged, mature megakaryocytes. No significant increase or left-shift of neutrophil granulopoiesis or erythropoiesis
2. Not meeting the WHO criteria for PV, PMF, CML, MDS or other myeloid neoplasm
3. Demonstration of JAK2V617F or other clonal marker or no evidence of reactive thrombocytosis
Minor Criteria
-
Diagnostic combinations
All 4 criteria must be met

Legend: CML = chronic myelogenous leukemia, MDS = myelodysplastic syndrome, PV = polycythemia vera, PMF = primary myelofibrosis.

Treatment: Low and intermediate risk patients with asymptomatic disease do not require therapy. Low-dose aspirin is an option. High-risk group needs the introduction of myelosuppressive therapy. It was proved that hydroxyurea is superior than anagrelide in preventing arterial thrombosis, although venous thrombosis was reduced in the anagrelide arm. It should be kept in mind that aspirin is contraindicated in extreme thrombocytosis due to acquired von Willebrand disease (38). Interferon-alpha is recommended for younger patients and in pregnancy, but this agent is often poorly tolerated. Anagrelide may effectively control platelet count especially in patients resistant or intolerant to hydroxyurea (34).

Prognosis: This is an indolent disease, but survival of ET patients is reduced by about 2-fold compared with general population. Major causes of death include thrombotic events and transformation to AML. Bone marrow fibrosis may develop as a disease termination, but it is rarely seen (5, 19).

PRIMARY MYELOFIBROSIS (PMF)

Definition: Primary myelofibrosis is a myeloproliferative neoplasm with an increased proliferation of megakaryocytes and granulocytes in the bone marrow associated with marrow fibrosis and extramedullary hematopoiesis. Initially, bone marrow is hypercellular with minimal deposition of fibrous connective tissue (prefibrotic phase). Subsequently, after several years of disease course, marrow picture evolves to a fibrotic phase with collagen fibrosis or even osteosclerosis (5).

Epidemiology: The incidence is estimated to occur at 0.5-1.5 per 100 000 persons per year. A median age at diagnosis is 6th-7th decade, but it may occur in young adults. Both genders are equally affected (39).

Clinical manifestations: About 30% of patients are asymptomatic at the time of diagnosis. The remaining

ones complain of fatigue, dyspnea, weight loss, night sweats, fever, thrombotic and bleeding events. Splenomegaly and hepatomegaly are detected in 90% and 50% respectively (5, 39).

Diagnostic criteria: see table 5 (5).

Table 5. WHO Diagnostic Criteria for Primary Myelofibrosis (5).

Major criteria
1. Megakaryocyte proliferation and atypia accompanied by either reticulin and/or collagen fibrosis or in the absence of reticulin fibrosis, the megakaryocyte changes must be accompanied by increased marrow cellularity, granulocytic proliferation and often decreased erythropoiesis (ie, pre-fibrotic cellular-phase disease)
2. Does not meet WHO criteria for CML, PV, MDS or other myeloid neoplasm
3. Demonstration of JAK2V617F or other clonal marker or no evidence of reactive marrow fibrosis
Minor Criteria
1. Leukoerythroblastosis
2. Increased serum LDH
3. Anemia
4. Palpable splenomegaly
Diagnostic combinations
All 3 major criteria and 2 minor criteria

Legend: CML = chronic myelogenous leukemia, MDS = myelodysplastic syndrome, LDH = lactate dehydrogenase, PV = polycythemia vera.

Risk stratification: There are three key prognostic scoring systems. The most used is "Lille score" which includes anemia (Hgb < 10g/dL) and abnormal leukocyte count (< 4 or > 30 x 10⁹/L). Based on these values we may distinguish three prognostic categories: low (no risk factors), intermediate (1 risk factor) and high (2 risk factors) with a median survival of 93, 26 and 13 months respectively (40). The new International Prognostic Scoring System (IPSS) uses 5 risk factors present at diagnosis: 1) age > 65 years, 2) hemoglobin < 10 g/L, 3) leukocyte count > 25 x 10⁹/L, 4) circulating blasts ≥ 1% and 5) the presence of constitutional symptoms. The presence of 0, 1, 2 and ≥ 3 factors defines low, intermediate-1, intermediate-2 and high risk disease with median survivals of 11.3, 7.9, 4 and 2.3 years, respectively (41). Recently, a Dynamic IPSS-plus scoring system (modified IPSS) was introduced for use at any time of disease course. This system includes three additional adverse risk factors: 1) red cell transfusion dependence, 2) platelet count ≤ 100 x 10⁹/L and 3) unfavorable cytogenetics. Using DIPSS-plus classification we may define low-risk category (no risk factors), intermediate-1 (1 risk factor), intermediate-2 (2 or 3 risk factors) and high (≥ 4 risk factors). Median survivals were 15.4, 6.5, 2.9 and 1.3 years, respectively (42). It was also demonstrated that presence or absence of molecular markers such JAK2, IDH and TET2 did not influence either survival and leukemic transformation whereas unfavorable karyotype or platelet count ≤ 100 x 10⁹/L did (43).

Treatment: The only therapeutic approach that has resulted favorably in survival is the application of allogeneic stem cell transplantation (allo-SCT), but one should keep in mind the high risk of death or chronic graft versus host disease (GVHD). Therefore, in every particular case one must consider risk/benefit ratio (43). Currently, it seems that this procedure should be reserved for high-risk patients and both myeloablative and reduced-intensity conditioning regimens have been used with similar efficacy. In patients who relapse after allo-SCT, a graft-versus-myelofibrosis effect could be demonstrated (44). Tefferi et al. (43) proposed to adjust the treatment to risk-groups. In asymptomatic patients with low or intermediate-1 DIPSS-plus score "watch and wait" strategy is recommended; whereas if these patients present symptomatic disease (it is unusual in this group), one of the conventional agents is preferred e.g. in anemic transfusion-independent patients with no splenomegaly and low serum erythropoietin level, the use of erythropoietin may result in a response rate of more than 50% (45). In the remaining cases one can choose between several agents including steroids, androgens, thalidomide and lenalidomide (43). PMF-patients with intermediate-2 or high risk category can be managed by allo-SCT (if fit enough), conventional and experimental agents, splenectomy and radiotherapy. All treatment modalities except of allo-SCT remain palliative. The use of lenalidomide is justified only in patients with del(5q) (46). It should be mentioned that several new agents are in clinical trials now including pomalidomide, hypomethylating agents and JAK inhibitors (43). The most promising is JAK inhibitor-CYT 387 with an 40% response rate in anemia, splenomegaly and constitutional symptoms (47). Myelofibrosis treatment algorithm was presented on figure 3 (43).

Prognosis: Life expectancy is 30% lower than in an age-matched and sex-matched population, with a median survival of 5 years (32). Major causes of death include thrombotic and bleeding events, infections, pulmonary hypertension and transformation to AML (48).

CONCLUSIONS

The breakthrough in the understanding of molecular basis of classical Philadelphia-negative MPNs was the identification of the JAK2V617F point mutation. It has changed dramatically our current view on disease pathogenesis and has direct implications in diagnostic algorithm as well as in therapeutic management. The 2008 WHO classification system for MPNs has integrated clinical, histological and genetic information and therefore facilitate the diagnostic strategy. A pivotal progress in molecular investigations we have witnessed, will certainly speed up further studies and subsequent revisions of WHO classifications are likely.

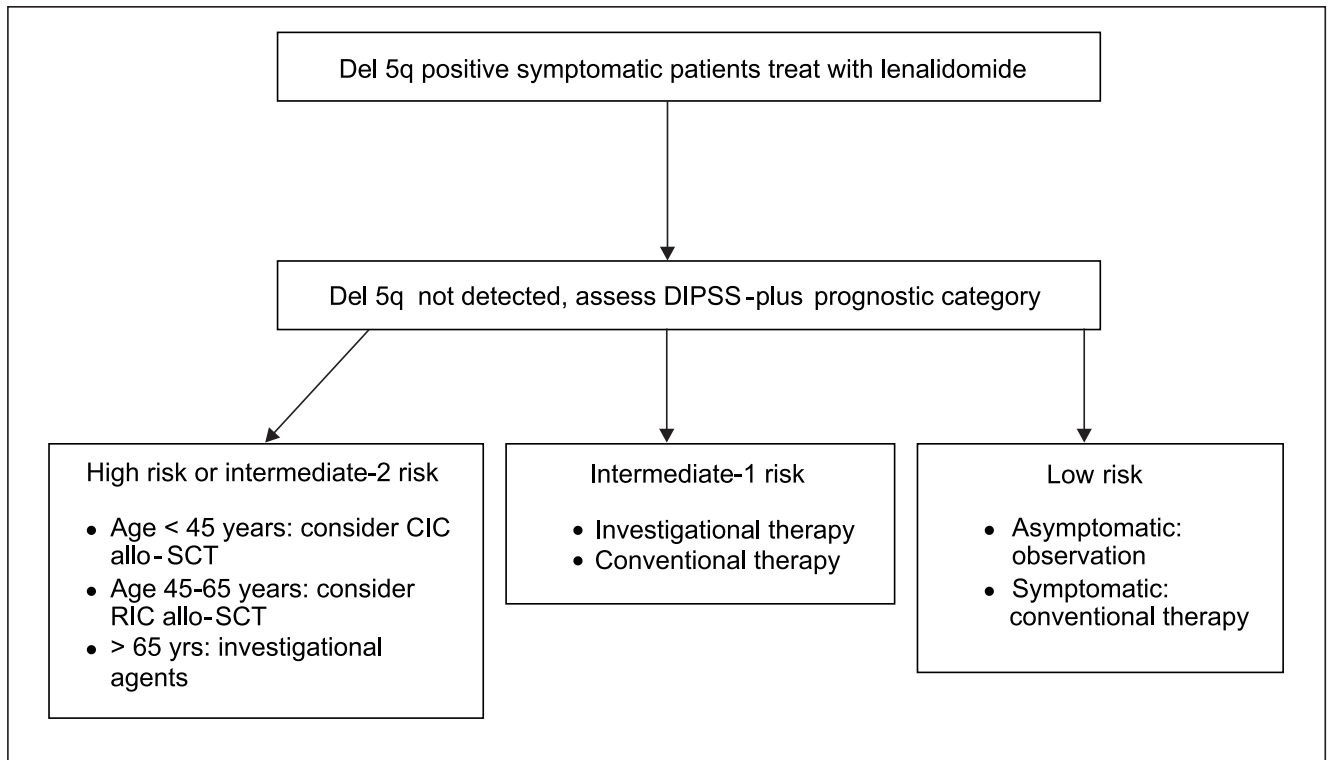


Fig. 3. Myelofibrosis treatment algorithm, modified by authors (43).

BIBLIOGRAPHY

- Vardiman JW, Brunning RD, Arber DA et al.: Introduction and overview of the classification of the myeloid neoplasms. [In:] Swerdlow SH, Campo E, Lee Harris N, et al. eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC Press 2008; 31-73.
- Dameshek W: Some speculations on the myeloproliferative syndromes. *Blood* 1951; 6: 372-375.
- Baxter EJ, Scott LM, Campbell PJ et al.: Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet* 2005; 365: 1054-1061.
- Tefferi A: Novel mutations and their functional and clinical relevance in myeloproliferative neoplasms: JAK2, MPL, TET2, ASXL1, CBL, IDH and IKZF1. *Leukaemia* 2010; 24: 1128-1138.
- Swerdlow SH, Campo E, Harris NL et al.: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC Press 2008.
- Murray PJ: The JAK-STAT signaling pathway: input and output integration. *J Immunol* 2007; 178: 2623-2629.
- Kilpivaara O, Levine RL: JAK2 and MPL mutations in myeloproliferative neoplasms: discovery and science. *Leukemia* 2008; 22: 1813-1817.
- Jones AV, Kreil S, Zoi K et al.: Widespread occurrence of the JAK2V617F mutation in chronic myeloproliferative disorders. *Blood* 2005; 106: 2162-2168.
- Kralovics R, Passamonti F, Buser AS et al.: A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med* 2005; 352: 1779-1790.
- Vanunucchi AM, Antonioli E, Guglielmelli P et al.: Prospective identification of high-risk polycythemia vera patients based on JAK2(V617F) allele burden. *Leukemia* 2007; 21: 1951-1959.
- Campbell PJ, Green AR: The myeloproliferative disorders. *N Engl J Med* 2006; 355: 2451-2466.
- Scott LM, Scott MA, Campbell PJ et al.: Progenitors homozygous for the V617F JAK2 mutation occur in most patients with polycythemia vera but not essential thrombocythemia. *Blood* 2006; 108: 2435-2437.
- Skoda R: The genetic basis of myeloproliferative disorders. *Hematology Am Soc Hematol Educ Program* 2007; 2007: 1-10.
- Campbell PJ, Baxter EJ, Beer PA et al.: Mutation of JAK2 in the myeloproliferative disorders: timing, clonality studies, cytogenetic associations and role in leukemic transformation. *Blond* 2006; 108: 3548-3555.
- Vannucchi AM, Antonioli E, Guglielmelli P et al.: Clinical profile of homozygous JAK2 617V > F mutation in patients with polycythemia vera or essential thrombocythemia. *Blood* 2007; 110: 840-846.
- Passamonti F, Rumi E, Pietra D et al.: A prospective study of 338 patients with polycythemia vera: the impact of JAK2(V617F) allele burden and leukocytosis on fibrotic or leukemic disease transformation and vascular complications. *Leukemia* 2010; 24: 1574-1579.
- Scott LM, Tong W, Levine RL et al.: JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. *N Engl J Med* 2007; 356: 459-468.
- Kaushansky K: The molecular mechanisms that control haematopoiesis. *J Clin Invest* 2005; 115: 3339-3347.
- Vannucchi AM, Guglielmelli P, Tefferi A: Advances in understanding and management of myeloproliferative neoplasms. *Ca Cancer J Clin* 2009; 59: 171-191.
- Beer PA, Campbell PJ, Scott LM et al.: MPL mutations in myeloproliferative disorders: analysis of the PT-1 cohort. *Blood* 2008; 112: 141-149.
- Guglielmelli P, Pancrazzi A, Bergamaschi G et al.: Anaemia characterizes patients with myelofibrosis harbouring Mpl mutation. *Br J Haematol* 2007; 137: 244-247.
- Tahiliani M, Koh KP, Shen Y et al.: Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by TET1. *Science* 2009; 324: 930-935.
- Delhommeau F, Dupont S, Della Valle V et al.: Mutation in TET2 in myeloid cancers. *N Engl J Med* 2009; 360: 2289-2301.
- Tefferi A, Levine RL, Lim KH et al.: Frequent TET2 mutations in systemic mastocytosis: clinical, KITD816F and FIP1L1-PDGFRα correlates. *Leukemia* 2009; 23: 900-904.
- Olcaydu D, Harutyunyan A, Jager R et al.: A common JAK2 hap-

- lotype confers susceptibility to myeloproliferative neoplasms. *Nat Genet* 2009; 41: 450-454.
26. Passamonti F, Rumi E, Pietra D et al.: JAK2 (V617F) mutation in healthy individuals. *Br J Haematol* 2007; 136: 678-679.
 27. Sidon P, El Housni H, Dessars B et al.: The JAK2V617F mutation is detectable at very low level in peripheral blood of healthy donors. *Leukemia* 2006; 20: 1622.
 28. Wadleigh M, Tefferi A: Classification and diagnosis of myeloproliferative neoplasms according to the 2008 World Health Organization criteria. *Int J Hematol* 2010; 91: 174-179.
 29. Marchioli R, Finazzi G, Landolfi R et al.: Vascular and neoplastic risk in a large cohort of patients with polycythemia vera. *J Clin Oncol* 2005; 23: 2224-2232.
 30. McMullin MF, Bareford D, Campbell P et al.: Guidelines for the diagnosis, investigation and management of polycythemia/erythrocytosis. *Br J Haematol* 2005; 130: 174-195.
 31. Finazzi G, Barbui T. How I treat patients with polycythemia vera. *Blood* 2007; 109: 5104-5111.
 32. Cervantes F, Passamonti F, Barosi G et al.: Life expectancy and prognostic factors in the classic BCR/ABL-negative myeloproliferative disorders. *Leukemia* 2008; 22: 905-914.
 33. Jensen MK, de Nully BP, Nielsen OJ et al.: Incidence, clinical features and outcome of essential thrombocythemia in a well defined geographical area. *Eur J Haematol* 2000; 65: 132-139.
 34. Harrison CN, Campbell PJ, Buck G et al.: Hydroxyurea compared to anagrelide in high risk essential thrombocythemia. *N Engl J Med* 2005; 353: 33-45.
 35. Gisslinger H: Update on diagnosis and management of essential thrombocythemia. *Semin Thromb Hemost* 2006; 32: 430-436.
 36. Carobbio A, Antonioli E, Guglielmelli P et al.: Leukocytosis and risk stratification assessment in essential thrombocythemia. *J Clin Oncol* 2008; 26: 2732-2736.
 37. Harrison C: Management of essential thrombocythemia: lessons from the Primary-Thrombocythemia 1 trial. *Haematologica* 2010; 4: 197-203.
 38. Budde U, Schaefer G, Mueller N et al.: Acquired von Willebrand's disease in the myeloproliferative syndrome. *Blood* 1984; 64: 981-985.
 39. Tefferi A: Myelofibrosis with myeloid metaplasia. *N Engl J Med* 2000; 342: 1255-1265.
 40. Dupriez B, Morel P, Demory JL et al.: Prognostic factors in agnogenic myeloid metaplasia: a report on 195 cases with a new scoring system. *Blood* 1996; 88: 1013-1018.
 41. Cervantes F, Dupriez B, Pereira A et al.: New prognostic scoring system for primary myelofibrosis based on a study of the International Working Group for Myelofibrosis Research and Treatment. *Blood* 2009; 113: 2895-2901.
 42. Gangat N, Caramazza D, Vaidya R et al.: DIPSS-Plus: a Refined Dynamic International Prognostic Scoring System (DIPSS) for Primary Myelofibrosis that incorporates prognostic information from karyotype, platelet count and transfusion status. *J Clin Oncol* 2010. Doi:10.1200/JCO.2010.32.2446.
 43. Tefferi A: How I treat myelofibrosis. *Blood* 2011; Doi: 10.1182/blood-2010-11-315614.
 44. Kroger N, Mesa RA: Choosing between stem cell therapy and drugs in myelofibrosis. *Leukemia* 2008; 22: 474-486.
 45. Huang J, Tefferi A: Erythropoiesis stimulating agents have limited therapeutic activity in transfusion-dependent patients with primary myelofibrosis regardless of serum erythropoietin level. *Eur J Haematol* 2009; 83: 154-155.
 46. Tefferi A, Lasho TL, Mesa RA et al.: Lenalidomide therapy in del(5)(q31)-associated myelofibrosis: cytogenetic and JAK2V617F molecular remissions. *Leukemia* 2007; 21: 1827-1828.
 47. Pardanani A, George G, Lasho T et al.: A Phase I/II Study of CYT 387, an oral JAK1/2 inhibitor, in myelofibrosis: significant response rates in anemia, splenomegaly and constitutional symptoms. *ASH Annual Meeting Abstracts*. November 20, 2010; 116: 460.
 48. Tefferi A: Primary myelofibrosis. *Cancer Treat Res* 2008; 142: 29-49.

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