MINISTRY OF HEALTH OF THE REPUBLIC OF MOLDOVA

"NICOLAE TESTEMITANU" STATE UNIVERSITY OF MEDICINE AND PHARMACY

Musteata Vasile, Robu Maria

PRIMARY MYELOFIBROSIS: CLINICAL PATTERNS, DIAGNOSIS AND TREATMENT

(Methodical guidelines for students)

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CONTENTS

LIST OF ABBREVIATIONS

PMF – primary myelofibrosis,
CD – cluster of differentiation,
BM – bone marrow,
JAK – Janus kinases,
FISH – fluorescence in situ hybridization,
CT – computed tomography,
ECOG – The Eastern Cooperative Oncology Group,
WHO – Word Health Organisation,
CML – chronic myeloid leukemia,
BCR-ABL1 – breakpoint cluster region-Abelson 1,
JAK2 – Janus kinase 2,
CALR – calreticulin,
MPL – myeloproliferative leukemia,
MPNs – myeloproliferative neoplasms,
PV – polycythemia vera,
ET – essential thrombocythemia,
LDH – lactate dehydrogenase,
RT-QPCR – reverse transcriptase quantitative polymerasechain reaction,
HSCT – hematopoietic stem cell transplantation,
OS – overall survival,
RFS – relapse free survival,
MChT– monochemotherapy

Definition

Primary myelofibrosis (PMF), also known as agnogenic myeloid metaplasia, represents a CMN, which emerges due to a clonal myeloid proliferation induced bystem cell malignant transformation. The disease is characterized by splenomegaly, BM fibrosis, anemia, extramedullary hematopoiesis, slow tendency to cachexia and blast transformation [1]. The first reported case of PMF was probably registered by Gustav Heuck in 1879, who described it as a 'peculiar leukaemia' [2]. It was not until Dameshek's original work in 1951 that PMF was recognized as a myeloproliferative neoplasm [3]. In 1951, Dameshek was the first to highlight the considerable overlap in the clinical and laboratory features of PV, ET and PMF, thus suggesting that these cover a range of related diseases along with CML. He coined the term "myeloproliferative disorders" to comprise these related conditions [4]. Over the last two decades, significant progress has been made in the diagnosis and treatment of PMF, especially related to the implementation of immunohistochemical, cytogenetic and molecular examinations, allogeneic stem cell transplantation and targeted chemotherapy. Nevertheless, the disease remains incurable in most cases, thus, significantly affecting patients' quality of life and life expectancy.

Preface

These methodical guidelines comprise the basic topics necessary for medical students that can assist in diagnosing PMF, as well as elaborating the treatment principles and personalized therapeutic strategies. The methodical guidelines constitute an effort to make the subject issues on PMF available, thus being perceived by the students. The major objective is to support the students of the Faculties of Medicine and Public Health. There have been defined the notions that will allow enriching the knowledge related to this field , as well as will provide useful data at all steps of knowledge assessment. An appropriate attention will be paid to the clinical activity of the students at the patient's bed, when carrying out practical skills, tests and clinical cases on the relevant subject. In order to achieve a better understanding of the subject issues presented in this methodical guidelines, we opted

for using graphical materials (tables, figures, and diagrams), images, clinical cases and assessments tests.

Duration of the seminar

The seminar lasts for 5 hours.

The aim of the seminar

To study the epidemiological patterns, etiology, pathogenesis, clinical features, laboratory tests and health care options applied for diagnosing PMF, synthesis of treatment principles and customized strategies.

Objectives of the seminar

1. To build-up knowledge on the epidemiology, etiology and pathogenesis of PMF;
2. To develop knowledge on the clinical, hematological, morphological, immunophenotyping, immunohistochemical, cytogenetic and molecular features of PMF;
3. To acquire practical skills in PMF diagnosis;
4. To build-up knowledge on differential diagnosis of PMF;
5. To develop general principles and personalized strategies of PMF treatment.

The seminar will be proceeded

1. On Hematology discipline, at "Nicolae Testemitanu" SUMPh, in groups of students, by using study modules.
2. Within the Hematology units at the Hematology Department of the PMSI Institute of Oncology of the Republic of Moldova.
3. Within the hematology offices at the Consulting Diagnostic Centre of the PMSI Institute of Oncology of the Republic of Moldova.

Methods and Materials used for the seminar

Teaching methods to be used

The teaching methods and procedures, involved in the effective learning and achievement of the suggested objectives, are as following:

- topic presentation by defining, describing, explaining and demonstrating;
- joint interactive discussion;
- problem-solving cases;
- data summarizing and synthesizing.

Various forms of independent, frontal, group, interactive activities are used during the seminars.

Methods of evaluation

- questioning on the study issue;
- problem-solving situations;
- analysis of clinical cases;
- single choice and multiple choice tests;
- individual work;
- assessment of practical skills;
- taking exam.

Materials used at the seminar

Teaching materials such as tables, schemes, algorithms, digital images, international guidelines are used for broader learning of PMF. Power Point presentations will be also applied during the seminars.

Questions for self-training of the student

1. Epidemiology and etiology of PMF.
2. Pathogenesis of PMF.
3. Clinical patterns, types and complications of PMF.
4. Diagnosis of PMF.
5. Hematological features of PMF.

6. The diagnostic value of bone marrow biopsy in PMF.
7. Prognostic scoring in PMF.
8. The diagnostic value of immunophenotyping and immunohistochemistry in PMF.
9. The diagnostic value of cytogenetic and molecular examinations in PMF.
10. Differential diagnosis of PMF.
11. Management principles and treatment options in PMF.
12. Evolution and prognosis of PMF.

Epidemiology of primary myelofibrosis

According to most literature data, PMF morbidity is 0.7-1.5 case per 100,000 population, with approximately equal gender involvement [5,6,7]. The median age of patients exceeds the corresponding indicator in CML, amounting to 65-70 years [8,9]; however, the disease may occur during the neonatal period up to the ninth decade of life [10]. The onset of PMF in children commonly starts within the first 3 years of life. In young children, girls are affected twice as often as boys are. In young and middle-aged adults, the disease is similar to that in the elderly, although the incidence of indolent cases may be higher. In 11% of cases, PMFis diagnosed in people under 45 years of age and in 22% of cases - under 55 years. Most studies estimate the median survival as being 3.5-5 years, ranging from one year in some patients to decades in others. Indeed, PMF is a heterogeneous disease from morphological, clinical and evolutionary characteristics, as well as due to its prognostic variability.

Etiology of primary myelofibrosis

The etiology of the disease has not been sufficiently studied so far. High-dose ionizing radiation, exposure to industrial chemicals (toluen and benzene derivatives) are reported as favouring factors of PMF development [11,12]. However, more data are required to establish a significant relationship due to a lownumber of reported cases.

Pathogenesis of primary myelofibrosis

Primary myelofibrosis is a clonal myeloproliferative neoplasm of the pluripotent haemopoietic stem cell, in which the proliferation of multiple cell lineages is accompanied by progressive BM fibrosis [3]. The BM fibrosis is thought to be secondary to the release of proinflammatory cytokines from abnormal clonal cells (primarily megakaryocytes), which act to stimulate fibroblast proliferation and fibrosis. Approximately 85–90% of PMF patients are carriers of mutations in JAK2, MPL (myeloproliferative leukemia) or CALR (calreticulin) genes, which are driver genes of overgrowth [1,13,14]. These mutations are not specific for PMF, being also determined in patients with PV and ET. Molecular and genetic studies have identified the JAK2-V617F somatic mutation in PMF patients, polycythemia vera and essential thrombocythemia in 2005 [13]. The mutation is formed in exon 14 of the JAK2 gene. The specialized literature data have pointed to the main pathogenetic mechanism, involving the JAK disorder (signal transducers) and transcriptional activators (JAK / STAT) of the signal transmission cascade *(Figure 1).* Ligand binding to cell surface cytokine receptors initiates auto phosphorylation of JAK2 and phosphorylation of cytoplasmic receptor tyrosines. STATs bind receptor phosphotyrosines via SH2 domains and translocate to the nucleus to induce expression of effector genes such as antiapoptotic Pim kinase and BclxL, cyclins promoting cell-cycle progression, and SOCS forming a negative feedback loop. JAK2 activates the PI3K/Akt and the MAPK pathways promoting proliferation and survival. Nuclear JAK2 is involved in epigenetic modifications [15]. There is increased signaling through JAK2 kinase transducers and STAT3 and STAT5 transcriptional activators. JAK2 kinase is involved in signaling from myeloid cytokine receptors. It attaches to homodimeric myeloid receptors, including the erythropoietin receptor, the thrombopoietin receptor (MPL), and the granulocyte colony stimulating factor receptor. The MPL gene is located on chromosome 1p34, encodes the thrombopoietin receptor and contributes to the growth and survival of megakaryocytes. W515 codon mutations activate the thrombopoietin receptor by stimulating the cytokine-independent JAK-STAT cascade. Medullary fibrosis results from the nonspecific reaction to malignant clonal activity, the fibroblasts being stimulated by PDGF, calmodulin, TGFB and VEGF. Family members of PMF patients have an increased risk of developing chronic myeloproliferative neoplasms due to the existence of predisposing chromosomal loci. Patients with triple ne-

gative PMF to JAK2, CALR and MPL mutations show a higher rate of blast transformation, followed by a poor prognosis.

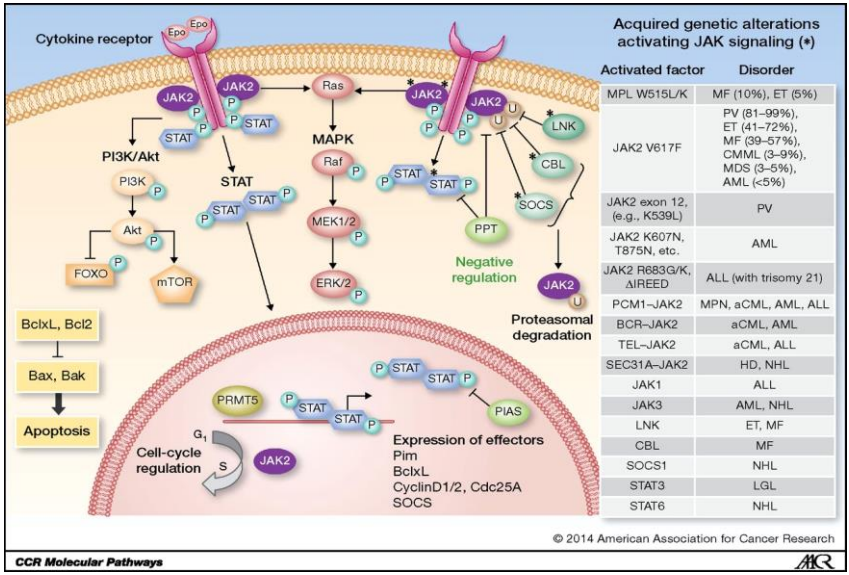

Figure 1. **Pathogenesis of PMF** [15]

Clinical features, types and complications of primary myelofibrosis

The clinical picture of PMF commonly varies. As in CML, approximately 30% of patients may be asymptomatic or oligosymptomatic when diagnosed, the disease being detected occasionally due to splenomegaly and changes in the blood count. Most patients present with splenomegaly or both splenomegaly and hepatomegaly, progressive anemia, constitutional (fever, night sweats) or hypercatabolism (weight loss, anorexia) symptoms [1,13,14]. An enlarged spleen is found in almost all patients at presentation. Splenomegaly can cause abdominal pain or abdominal discomfort. Spleen pain and discomfort are the common presenting symptoms of PMF. Most patients develop moderate to marked splenomegaly during the course of the disease and about 10% of them develop massive splenomegaly, with the spleen extending to the right iliac fossa. This huge increase in spleen mass (up to 20–30 times normal) can lead to a significant increase in splenic blood flow, which can result in portal hypertension

with esophageal varices and ascites in most severe cases. Painful and painless splenic infarcts are common sequelae of splenomegaly in PMF. These may be accompaned by headaches, fatigue, peripheral edemas, ascites, itchy skin, bone pain, thromboses, spinal infarction. A hypermetabolic condition associated with fevers, anorexia, weightloss and night sweats develops in most PMF cases, sometimes inthe early onset of the disease. The presence of these symptoms is associated with poor patient prognosis. These symptoms significantly reduce the patients' quality of life. The pathology gradually evolves from the prefibrotic phase to the fibrotic phase with the progressive development of medullary failure.

Diagnosis of primary myelofibrosis

The diagnosis of PMF is based on the results of histopathological examination of the BM *(Figure 1, 2)* and molecular-genetic investigations for the detection of JAK2, MPL or CALR mutations. The WHO Working Group on Myeloproliferative Neoplasms identified major and minor diagnostic criteria for the prefibrotic and fibrotic phase of PMF *(Table 1)* [11,14].

Table 1 **The 2016 WHO diagnostic criteria for prefibrotic and fibrotic phase of PMF [11,14]**

	PrePMF	PMF
Major criteria	1. Megakaryocytic proliferation and atypia, without reticulin fibrosis > grade 1, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation and often decreased erythropoiesis 2. Not meeting the WHO criteria for BCR-ABL1+ CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms 3. Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these mutations, presence of another clonal marker or absence of minor reactive BM reticulin fibrosis	1. Presence of megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3 2. Not meeting WHO criteria for ET, PV, BCR-ABL1+ CML, myelodysplastic syndromes, or other myeloid neoplasms 3. Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these mutations, presence of another clonal marker or absence of reactive myelofibrosis
Minor criteria	Presence of at least 1 of the following, confirmed in 2 consecutive determinations: a. Anemia not attributed to a comorbid condition b. Leukocytosis > 11×10^9/L c. Palpable splenomegaly d. LDH increased to above upper normal limit of institutional reference range	Presence of at least 1 of the following, confirmed in 2 consecutive determinations: a. Anemia not attributed to a comorbid condition b. Leukocytosis > 11×10^9/L c. Palpable splenomegaly d. LDH increased to above upper normal limit of institutional reference range e. Leukoerythroblastosis
Diagnosis		All three major criteria, and at least one minor criterion

Hematological features of primary myelofibrosis

Relevant changes in the blood count include moderate leukocytosis, with left deviation to the myelocytes, metamyelocytes, solitary blast cells. Hemoglobin levels and erythrocyte counts may be low, within normal limits, or rarely increased (Vaughan type). Complete blood count reveals.

Adapted from Arber et al., with the permission of American Society of Hematology variable leukocytosis ($\geq$25 x 10^9/l – in 10% of cases) or leukopenia (<3 x 10^9/l – in 10% of cases). Platelet count ranges from thrombocytopenia (40% of cases) to hyperthrombocytosis ($\geq$1000 x 10^9/l). The presence of erythroid and myeloid precursors in the peripheral blood (leucoerythroblastic picture) is common. Anisocytosis and poikilocytosis of erythrocytes and erythrocaryocytes may occur. Teardrop poikilocytes (dacryocytes), basophilic stippling, macrocytosis (related to factitious folate deficiency), giant platelets and megakaryocyte fragments may also be detected.

The diagnostic value of bone marrow aspiration and biopsy in primary myelofibrosis

BM aspiration may yield a dry-tap or a haemodilute sample, making aspirate morphology of limited diagnostic value [3]. Sufficient material can often be obtained from either BM or peripheral blood to assess the karyotype, which can help exclude diagnoses such as CML. BM trephine biopsy is essential for PMF diagnosis. Early stages are characterized by BM hypercellularity due to the excessive proliferation of granulocyte, erythrocaryocyte and megakaryocyte cell lines, associated with a disorganization of marrow architecture and the presence of abnormal large megakaryocytes often occurring in clusters [16]. In some marrow cavities, especially in the fibrotic phase, fibrosis is characterized by the increased deposition of reticulin fibers and collagen fibers**.** BM fibrosis becomes increasingly dominant and progressively replaces hematopoiesis. Intrasinusoidal hemopoiesis may sometimes be observed at this stage.

Prognostic scoring in primary myelofibrosis

Prognostic score calculation systems are developed for PMF patients. The International Prognostic Scoring System (IPSS), designed in 2009, describes 5 risk factors to estimate the patient survival: the presence of

constitutional symptoms, over 65-year age, hemoglobin level below 10 g/dL, leukocyte count over 25 x 10^9/l, percentage of circulating blast cells ≥ 1% *(Table 2, 3)* [14]. In 2011, the DIPSS-plus system was developed to integrate data on the need of transfusions of erythrocyte concentrate, unfavorable karyotype and platelet count below 100 x 10^9/l. Patients were classified into 4 risk groups: low risk (no risk factors), intermediate risk-1 (1 risk factor), intermediate risk-2 (2-3 risk factors) and high risk (≥4 factors risk), the median OS being equivalent to 185, 78, 36 and 16 months, respectively.

The diagnostic value of immunophenotyping and immunehistochemistry in primary myelofibrosis

Recent studies have revealed that the abnormalities in frequency and function of blood cells manifested by an alteration in CD markers' expression features play a key role in thrombo-hemorrhagic, autoimmune or inflammatory complications [17]. Since JAK2 V617F mutation affects the

Table 2

International Systems of Prognostic Scoring in primary myelofibrosis [6, 10, 12]

Variable	IPSS [2]	DIPSS [29]	DIPSS plus [30]
Age > 65 years	√	√	√
Constitutional symptoms[a]	√	√	√
Hb < 10 g/dL	√	√	√
WBC > 25,000/μL	√	√	√
Peripheral blood blasts ≥ 1%	√	√	√
Platelets < 10 × 10^4/μL			√
Red cell transfusion need[b]			√
Unfavorable karyotype[c]			√
Point per variable	1 point each	1 point each but Hb = 2	1 point each

IPSS, International Prognostic Scoring System; DIPSS, Dynamic IPSS; DIPSS plus, Dynamic IPSS plus additional prognostic factors; Hb, hemoglobin; WBC, white blood cell.

[a]Weight loss 10% of the baseline value in the year preceding primary myelofibrosis diagnosis and/or unexplained fever or excessive sweats persisting for more than 1 month.

[b]Red blood cell (RBC) transfusion at the time of referral and those with history of RBC transfusions, for myelofibrosis-associated anemia.

[c]Complex karyotype or single or tow abnormalities including +8, -7/7q-, i(17q), -5/5q-, 12p-, inv(3), or 11q23 rearrangements.

Table 3

Risk stratification in primary myelofibrosis in regard to the International System of Prognostic Scoring [5,14,16]

Risk group	IPSS [2]		DIPSS [29]		DIPSS plus [30]	
	No. of factors	Median survival, yr	No. of factors	Median survival, yr	No. of factors	Median survival, yr
Low	0	11.3	0	Not reached	0	15.4
Intermediate-1	1	7.9	1, 2	14.2	1	6.5
Intermediate-2	2	4.0	3, 4	4	2, 3	2.9
High	≥ 3	2.3	5, 6	1.5	≥ 4	1.3

IPSS, International Prognostic Scoring System; DIPSS, Dynamic IPSS; DIPSS plus, Dynamic IPSS plus additional prognostic factors.

CD110 expression, the reduced expression of this marker in JAK2 V617F-positive MPNs may be a poor prognostic factor for increased thrombopoiesis and subsequently enhance the risk of thrombotic complications. Contrastingly to CD110, the expression of P-selectin (CD62P) as an activation marker of immature platelets is higher in MPNs patients. CD62P is a surface glycoprotein mediating the interaction of activated platelets with endothelial cells. However, JAK2 V617F mutation has been demonstrated to be significantly associated with the increased frequency of these immature cells in PMF. Platelet activation occurs by changing expression patterns of some functional CD markers. The changing expression of other platelet functional markers, including CD63 and CD154 (soluble CD40 ligand), has been observed on platelets of JAK2 V617F-positive MPNs, both of which can be prognostic for thrombotic complications in PMF. In JAK2 V617F-positive MPNs, an increase in circulating endothelial cells is observed in addition to changing function of these cells [18]. Endothelial cells are characterized by the expression of CD34 and CD133 in their precursors, as well as CD309 (vascular endothelial growth factor receptor) and CD31 (platelet and endothelial cell adhesion molecule 1) expression in their mature form. The increase in circulating endothelial cells has a direct relationship with angiogenesis in MPNs and especially in PMF. Different subtypes of lymphoid cells, including $TCD4^{+}$ and $TCD8^{+-}$ cells, are considerably reduced in PMF patients compared with healthy subjects. Contrarily, a small number of these patients exhibit an increase in B $CD5^{+}$ and T $CD8^{+}$ cytotoxic lymphocytes [19]. Activated leukocytes (such as neutrophils and monocytes) can be involved in the development

of arterial and venous thromboses through their interaction with platelets and endothelial cells, which are mediated by the expression of integrins like CD11c and CD11b on leukocytes. Several studies demonstrated the increased expressions of these integrins as well as CD14 increase on monocytes and neutrophils of in PMF patients. Another PMF feature is the expansion and activation of monocyte-macrophage system, which is characterized by a considerable increase in mature macrophages in BM and monocytes in peripheral blood. In PMF patients, CD68-positive monocytosis has been shown to be associated with the onset of accelerated phase of the disease, while patients showing this feature are considered as high-risk group.

The diagnostic value of cytogenetic and molecular examinations in primary myelofibrosis

The following investigations are mandatory for diagnosis and differential diagnosis of PMF: cytogenetic and molecular examinations of BM aspirate, venous blood with determination of molecular-genetic markers of tumor clones by FISH, nested / multiplex PCR or RT-PCR (p210 transcript of BCR-ABL fusion gene, quantitative detection of JAK2 V617F mutation, detection measurement of MPL mutation, detection of trisomy 9 or del (13q)). Approximately 85% of patients with PMF are the carriers of driver gene mutations in transcription (JAK/ STAT) signaling pathways [14], including JAK2V617F [20], MPLW515 [21], and calreticulin (CALR) [22]. There may be additional mutations in epigenetic regulators and RNA splicing genes that co-exist with driver mutations and play critical roles in disease progression [23, 24, 25]. Most PMF patients are likely to carry one out of three mutually exclusive somatic driver mutations [14]; JAK2V617F in up to 60%, CALR in up to 20%, and MPL in up to 5% of patients. Additional non-driver mutations associated with epigenetic modification (TET2, ASXL1, EZH2, IDH1/2), RNA splicing (SRSF2, SF3B1, U2AF1 [U2 small nuclear RNA auxiliary factor 1]), JAK/STAT signaling (CBL [Casitas B-cell lymphoma], LNK), and DNA repair (TP53 [tumor protein p53]) are revealed in 1-10% of patients. In cytogenetic analyses, chromosomal abnormalities such as complex karyotypes and single or double abnormalities, including +8, del(7)/7q-, 12p-, inv (3), and 11q23 rearrangements, are defined as unfavorable karyotypes [14,26].

Differential diagnosis of primary myelofibrosis

The differential diagnosis of PMF should be carried out with other pathologies that can evolve with splenomegaly, leukocytosis or leukopenia, thrombocytosis or thrombocytopenia and deviation of the leukocyte formula to the left [3]:

- other CMNs (chronic phase of CML, PV, ET);
- Acute megakaryoblastic leukemia;
- Myelodysplastic syndromes;
- Malignant lymphomas with splenomegaly and metastatic involvement of the BM (Hodgkin's lymphoma, non-Hodgkin's lymphomas);
- Metastatic carcinoma;
- Inflammatory and infectious diseases (Paget's disease, tuberculosis).

The main pathological feature of ET is a persistent increase of the platelet count without a left shift in the leukocyte formula. Splenomegaly is present in about 5% of ET patients upon diagnosis and is commonly mild. A progressive spleen enlargement during the course of ET may raise suspicion of a developing myelofibrosis. The presence of a JAK2V617F, CALR or MPLmutations provide useful positive diagnostic criteria for approximately 80–90% of ET patients. BM histological features such as giant, multilobated megakaryocytes and megakaryocyte clustering might be valuable in diagnosing ET.

The most important pathological feature of PV is an expansion of the total red cell mass, although elevations in the platelet and/or neutronphil counts are relatively common. Palpable splenomegaly is observed in 30–50% of PV cases. Plethora, dilated conjunctival vessels and rosacea-like facial skin changes are common at presentation. Most patients can be diagnosed based on elevated hematocrit levels (>0.52 in men and >0.48 in women) along with the presence of the *JAK2* V617F mutation. The role of the BM biopsy in PV evaluation remains controversial, though it may be useful as a baseline investigation for later comparison in younger diseased patients, or in case of unusual clinical and laboratory features at presentation.

The most frequent symptoms of CML chronic phase include progressive splenomegaly, weight loss, nightsweats and fatigue. The most characteristic hematological abnormality is marked leukocytosis (higher than in PMF) with left shift in the leukocyte formula. BN is commonly hypercellular due to an extremely increased myeloid activity. Myeloid maturation

is left shifted, and blasts are usually less than 5%. The diagnosis of CML is based on detection of Philadelphia chromosome via the cytogenetic analysis, or BCR–ABL1fusion gene with RT-QPCR in terms of compatible blood and marrow morphology.

Principles of management and treatment options in primary myelofibrosis

The current PMF treatment is focused on increasing the survival rate and patients'life quality by preventing and combating thromboembolic and hemorrhagic complications and monitoring the systemic symptoms. International guidelines and published studies show that allogeneic BM transplantation is the treatment of choice inpatients under 60 years old, which can considerably increase the patients' lifespan, as well as their recovery rate [1,9,14]. The literature data have revealed that the 3-year OS and 5-year RFS have been recorded in 39% and 22 - 33% of cases, respectively. At the same time, BM transplantation in PMF is reported to be associated with 50% of deaths and related morbidities.

The therapeutic value of asymptomatic or oligo symptomatic patients with low or intermediate risk-1 is considered questionable. Treatment might be indicated for anemia, massive splenomegaly, bone pain, pulmonary hypertension, constitutional symptoms, hyperleukocytosis and thrombocytosis. Hydroxycarbamide (2-3 g daily) is administered in patients with thrombocytosis. In cases with marked symptoms, especially anemia, Thalidomide (50 mg daily) may be given in combination with Prednisolone (0.5 mg/kg daily) or Lenalidomide (10 mg daily) in combination with Prednisolone (0.25-0,5 mg/kg daily), androgen hormones, Danazol (600 mg daily), transfusions of deleukocytized or deplasmatized erythrocyte concentrate. Higher efficacy of Lenalidomide has been reported in patients with del (5q31). Hydroxycarbamide MChT (20-30 mg/kg/day) is used in patients with splenomegaly and thrombocytosis as a first-line treatment. Interferon-α can induce molecular remissions in JAK2-positive chronic MPNs with no teratogenic effects and is preferable in patients under the age of 60 as a second-line therapeutic option. Induction therapy of α-Interferon is 3 MIU x 3 times/week with the maintenance of the minimum dose-response that keeps the hemoleukogram values within the normal limits. Splenectomy is indicated in cases of massive splenomegaly that is unresponsive to drug treatment and if associated with portal hypertension and hemolytic anemia.

Recombinant human erythropoietin may be used to treat anemia. According to current literature sources, a response to erythropoietin is registered in approximately 30-50% of patients, especially in cases with a plasma EPO concentration below 125 mU/mL and a monthly rate of erythrocyte concentrate transfusions of no more than 3 units [27,28]. The initial dose of Erythropoietin is 600 U/kg per week (maximum dose 40 000 U per week).

Patients with intermediate-2 or high risk, especially with ASXL1 and SRSF2 prognostic adverse mutations, should be considered for allogeneic BM transplantation options or JAK kinase inhibitors (Ruxolitinib, Momelotinib). The recommended starting dose of Ruxolitinib in the treatment of PMF is 15 mg twice daily for patients with platelet counts between 100 – 200 x 10^9/l, and 20 mg twice daily for patients with platelets over 200x10^9/l. The induction dose of Momelotinib is 300 mg daily.

In cases of transformation into the acute phase, namely the blast crisis, the treatment refers to the morphocytochemical type of acute leukemia.

If autoimmune complications occur (autoimmune haemolytic anemia, autoimmune thrombocytopenia) glucocorticoids may be indicated (Prednisolone 1 mg/kg daily, Methylprednisolone, etc.).

Allopurinol is recommended to be administered at a dose of 200-600 mg daily for preventing and managing the development of nephrolithiasis and gouty arthritis.

Evolution and prognosis

The OS rate of patients with PMF largely varies, ranging from minimum 1-2 years up to 20-30 years, under the drug management in low-risk or intermediate risk-1cases. According to the international literature data, the OS in PMF (averaging 5.9 years) is lower than this corresponding indicator in other chronic Ph-negative MPNs such as PV (averaging 13.5 years) and ET (averaging 19.8 years) [12]. The transformation into acute phase, defined as a persistent presence of 20% blasts in blood or BM, occurs in 20–30% of cases of PMF and is commonly rapidly fatal. Current research findings suggest that patients with CALR mutations may have fewer thrombotic events with more myelofibrotic transformations, while those patients having any one of ASXL-1, EZH2, IDH1/2, and SRSF2 mutations appear to have aworse overall prognosis.

Clinical case studies

Clinical case 1

A 70- year-old woman presented with general weakness, discomfort and pain in the left upper abdominal quadrant, paleness of the face. She has been considered ill for two years. She was treated from chronic hepatitis by a therapist.

Physical exam: Severe patient's condition. ECOG-WHO score – 3. Paleness of skin. Impalpable peripheral lymph nodes. Lung exam: a vesicular murmur is determined. Heart sounds are rhythmical and quiet. Apical systolic murmur is detectable. Pulse rate is 86 beats/min. Blood pressure is 120/75 mm/Hg. The liver is palpable +3 cm below the costal margin. The spleen is palpable at the umbilical line.

Peripheral blood count: Hb – 98 g/l, RBCs – $3.2x10^{12}$/l, WBC – $19.5x10^{9}$/l, reticulocytes – 47‰, PLT – 528.0 $x10^{9}$/l, promyelocytes – 3%, myelocytes – 37%, metamyelocytes – 4%, bands – 12%, segm. – 35%, eos. – 2%, bas. – 4%, lymph. – 1%, mon. – 2%, erythrocyte sedimentation rate – 15 mm/h.

Biochemical test: unconjugated bilirubin – 25 mcmol/l, total bilirubin – 29 mcmol/l, LDH – 587 U/H, the other parameters are within the normal limits.

Bone marrow aspiration: Hypercellular. Myeloid cell line – 81%. An increased mumber of megakaryocytes. The erythroid cell lineage exhibits signs of megaloblastic hematopoiesis.

Abdominal ultrasound: Splenomegaly: 26x12 cm, homogeneous structure. Hepatomegaly: RL 17 cm, LL 11 cm, diffuse changes.

1. What diagnosis would you establish?
2. Could you develop an investigation plan?
3. What treatment plan is suitable for this case study?

Clinical case 2

A 52- year- old man presented with periodical headaches, left upper abdominal quadrant discomfort, pain in the fingers and thumbs, paleness of the face. The patient has been considered ill for one year and the above-mentioned sings gradually appeared. His condition has worsened over the last 3 months.

Physical exam: ECOG-WHO score 2. Face, arms and upper torso are slightly pale. Lung exam: a vesicular murmur is determined. Heart sounds are rhythmical and quiet. Pulse rate is 96 beats/min. Blood pressure – 100/70 mm/Hg. The liver is palpable at the level of costal margin. The spleen is palpable +4 cm below the left costal margin.

Peripheral blood count: Hb – 110 g/l, RBCs – $3.8x10^{12}$/l, WBCs – $14.2x10^{9}$/l, reticulocytes –12‰, PLT – 520.0 $x10^{9}$/l, myelocytes – 3%, metamyelocytes – 4%, bands – 5%, segm. – 63%, eos. – 2%, bas. – 1%, lymph. – 10%, mon. – 4%, erythrocyte sedimentation rate – 26 mm/h.

Biochemical test: LDH – 537 U/H, all other parameters are within the normal limits.

Bone marrow aspiration: Hypercellular. Myeloid cell line – 83%. An increased number of megakaryocytes.

Abdominal ultrasound: Splenomegaly: 18.5x9 cm, homogeneous structure. Liver: RL 14.5 cm, LL 7 cm, diffuse changes.

1. What diagnosis would you determine?
2. Could you develop an investigation plan?
3. What is the differential the diagnosis for this case study?
4. What is the treatment plan?

Clinical case 3

A 41-year-old man presented with fatigue, left upper abdominal quadrant discomfort and paleness of the face. The patient has been considered ill for one year, with gradual appearance of the above-mentioned sings. His condition has worsened over the last 2 months.

Physical exam: ECOG-WHO score 1. Face, arms and upper torso are slightly pale. Lung exam: a vesicular murmur is determined. Heart sounds are rhythmical and quiet. Pulse rate is 92 beats/min. Blood pressure – 110/70 mm/Hg. The liver is palpable at the level of costal margin. The spleen is palpable +3 cm below the left costal margin.

Peripheral blood count: Hb – 115 g/l, RBCs – $3.8x10^{12}$/l, WBC – $12.2x10^{9}$/l, reticulocytes – 12‰, PLT – 480.0 $x10^{9}$/l, myelocytes – 3%, metamyelocytes – 4%, bands – 5%, segm. – 63%, eos. – 2%, bas. – 1%, lymph. – 10%, mon. – 4%, erythrocyte sedimentation rate – 21 mm/h.

Biochemical test: all parameters are within the normal limits.

Bone marrow aspiration: Hypercellular, myeloid cell line – 79%. Number of megakaryocytes is increased.

Pyrosequencing and droplet digital PCR of the venous blood: quantitative detection of JAK2 V617F mutation – 24.7%.

Abdominal ultrasound scan: Splenomegaly: 17.5x9 cm, homogeneous structure. Liver: RL 14.5 cm, LL 7 cm, diffuse changes.

1. What diagnosis would you determine?
2. Could you develop an investigation plan?
3. What is the differential the diagnosis for this case study?
4. What is the treatment plan?

Assessment tests

S. The main symptom of primary myelofibrosis is:

A. Central nervous system involvement
B. Splenomegaly
C. Lymph nodes enlargement
D. Bone pain
E. Involvement of pulmonary tissue

Correct answer: **B**

S. In primary myelofibrosis, anemia may be of the following etiology:

A. Iron-deficiency
B. Renal
C. Vitamin B_{12}- deficiency
D. Autoimmune hemolytic
E. Heteroimmune hemolytic

Correct answer: **D**

S. In primary myelofibrosis, the erythrocyte morphology reveals:

A. Normocytic red cells
B. Anisocytosis and poikilocytosis
C. Hypochromia
D. Microspherocytes
E. Targeted red cells

Correct answer: **B**

S. In primary myelofibrosis, the cytogenetic and molecular examinationsmight detect:

A. BCR-ABL fusion gene transcripts
B. JAK2 V617F mutation
C. Translocation t(15;17)(q22;q21)
D. Translocation t(9;22)(q34;q11)
E. Targeted red cells

Correct answer: **B**

S. In primary myelofibrosis, mutation occurs within the:

A. Stem cell
B. Blast cells

C. Precursor cell of myelopoiesis
D. Precursor cell of B-lymphopoiesis
E. Precursor cell of T-lymphopoiesis

Correct answer: **A**

S. Which method of investigation is more informative in diagnosing primary myelofibrosis:

A. Sternal puncture
B. Complete blood count
C. Hemoglobin electrophoresis
D. Trepanobiopsia with histological study of bone marrow
E. Coagulogram

Correct answer: **D**

S. In primary myelofibrosis, splenectomy can be carried out in case of:

A. Complication with autoimmune hemolytic anemia and/or autoimmune thrombocytopenia
B. Hyperthrombocytosis
C. Hyperleukocytosis
D. Erythrocytosis
E. In none of the cases

Correct answer: **A**

S. The incidence of primary myelofibrosis per 100000 of population is:

A. 2.2
B. 0.8
C. 1.5
D. 1.9
E. 0.2

Correct answer: **E**

S. Which of the following statements confirms the diagnosis of primary myelofibrosis:

A. Absence of splenomegaly
B. Basophil-eosinophil association
C. Unchanged leukocyte alkaline phosphatase activity

D. Proliferation of two or three hematopoietic cellular lines with myelofibrosis

E. Absence of myelofibrosis

Correct answer: **D**

S. The main clinical feature suggesting the presence of primary myelofibrosis is as following:

A. The spleen is not enlarged

B. Pancytopenia in blood count

C. The spleen size does not correlate with the leukocyte count, and the left-side deviation of differential leukocyte count is moderate

D. Absence of poikilocytosis

E. Platelets are unchanged

Correct answer: **C**

C. The following statements are true for the diagnosis of primary myelofibrosis:

A. Absence of splenomegaly

B. Erythrocytes are normocytic

C. Anemia is uncommon

D. Thrombocytopenia is rarely observed

E. The platelet count is commonly increased

Correct answer: **D, E**

C. In primary myelofibrosis, histological examination of the spleen sections reveals:

A. Plasmatic cells

B. Myeloid line cells

C. Erythrocaryocyte and megacaryocytes

D. Lymphocytosis

E. Monocytosis

Correct answer: **B, C**

C. The diagnosis of primary myelofibrosis is confirmed by:

A. Leukocytosis over 100.0×10^9/l

B. Decreased values of alcaline phosphatase in neutrophils

C. Giant splenomegaly

D. Presence of Ph chromosome

E. Myeloid and megacaryocytic proliferation in the bone marrow biopsy sample

Correct answer: **C, E**

C. In primary myelofibrosis, splenectomy is indicated in the following cases:

A. Severe hypersplenism

B. Repeated splenic infarctions

C. The first-line therapeutic option in myeloproliferation phase

D. In marked bone marrow sclerosis

E. Autoimmune hemolytic complications

*Correct answer:***A, B, E**

C. The terminal phase of polycythemia vera is manifested by:

A. Posterythremic myelofibrosis

B. Acute leukemia

C. Chronic lymphocytic leukemia

D. Chronic myeloid leukemia

E. Sarcoma development

Correct answer: **A, B, D**

C. The following statements are true for the diagnosis of primary myelofibrosis:

A. Marked shift to the left of the leukocyte count, with high percentage of myelocytes and metamyelocytes

B. Morphological examination of erythrocytes reveals marked anisocytosis and poikilocytosis

C. Neutrophil alcaline phosphatase is normal or increased

D. Anemia is common

E. There is no correlation between leukocyte count and spleen size

Correct answer: **B, C, D, E**

C. The evolution of primary myelofibrosis may be associated with following complications:

A. Uricemia, nephrosclerosis

B. Portal hypertension

C. Transformation in acute leukemia
D. Megaloblastic hematopoiesis due to folic acid deficiency
E. Splenic infarctions

Correct answer: **A, B, C, E**

C. In primary myelofibrosis, splenectomy is contraindicated in the following cases:
A. Development of disseminated intravascular coagulation syndrome
B. Concomitant marked liver enlargement
C. Terminal stage of the disease, with accelerated spleen enlargement, cytopenia, fever and bone pain
D. Marked leukocytosis and thrombocytosis
E. Autoimmune hemolytic anemia

Correct answer: **A, B, C, D**

C. Which of the following leukemias are referred to the myeloproliferative processes:
A. Chronic myeloid leukemia
B. Acute lymphoblastic leukemia
C. Idiopathic myelofibrosis
D. Essential thrombocythemia
E. Erythremia

*Correct answer:***A, C, D. E**

C. Which of the following leukemias might develop from the myeloid cell precursor:
A. Chronic myeloid leukemia
B. Idiopathic myelofibrosis
C. Erythremia
D. Polycythemia vera
E. Agnogenic myeloid metaplasia

Correct answer: **B, C, D, E**

C. Stage III erythremia is also called:
A. Blast crisis
B. Sarcomatization
C. Blast transformation
D. Anemic
E. Posterythremic myelofibrosis

Correct answer: **D, E**

C. Which of the following drugs may be administered in primary myelofibrosis:

A. Lenalidomide
B. Interferon-α
C. Imatinib mesylate
D. Ruxolitinib
E. ATRA

Correct answer: **A, B, D**

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