Diagnosis of myelodysplastic syndromes in Poland: Polish Adult Leukemia Group (PALG) 2021 recommendations

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Abstract

Myelodysplastic syndromes (MDS) are a heterogeneous group of neoplastic diseases of the hematopoietic cells manifested by ineffective hematopoiesis and a tendency to transform into acute myeloid leukemia. MDS should be considered in the differential diagnosis of cytopenia, especially in the elderly. This article presents the recommendations of MDS experts of the Polish Adult Leukemia Group (PALG) for the diagnosis of myelodysplastic syndromes. We present current classifications and prognostic indices, as well as diagnostic examinations recommended for MDS: cytological, histopathological, immunophenotypic, cytogenetic and molecular tests. The aim of the study is to implement up-to-date knowledge about myelodysplastic syndromes into routine clinical practice, from the diagnosis of cytopenia to the specific diagnosis and prognosis in MDS patients.

Key words: myelodysplastic syndromes, diagnosis, recommendations
Introduction
Myelodysplastic syndromes (MDS) are neoplastic diseases with a variable clinical course originating from hematopoietic stem cells (HSCs). A characteristic feature of MDS is ineffective hematopoiesis manifested by dysplasia of bone marrow and peripheral blood cells, peripheral cytopenia, and an increased risk of transformation into acute myeloid leukemia (AML).

Epidemiology
It is estimated that the incidence rate of newly diagnosed MDS is 4.5/100,000/year. Usually the disease affects the elderly. The median adult age at diagnosis is 70–75 years, whilst according to the Polish MDS Registry it is 70 years [1, 2]. In the age bracket of 70–74, the incidence is 16.6/100,000/year; in the age bracket of 75–79 years it is 25.7/100,000/year; and this increases to over 36/100,000/year in people aged 80 and over [3]. MDS in children is very rare, with a median age at diagnosis of 6.8 years. In the population of people under 30 years, the incidence rate of newly diagnosed MDS is 0.1/100,000/year. Patients <50 years of age constitute 9% of all MDS cases. The disease is more prevalent among men, with a male to female ratio 1.1–1.4:1 and this ratio increases with age.

The number of MDS cases is probably underestimated due to the lack of a proper diagnosis of cytopenia in the elderly.

Clinical manifestation
The clinical manifestation of MDS is not characteristic, with symptoms most often resulting from cytopenia. There are features of anemia, thrombocytopenia, infections (usually bacterial and fungal) resulting from granulocytopenia and dysfunction of neutrophils. Hepato- and splenomegaly is rare. General symptoms (weight loss, fever, sweating) are also not common and occur in higher risk MDS patients or during leukemic transformation. Autoimmune diseases are detected in c.19–28% of MDS patients [4], and the most common include hypothyroidism (12%), rheumatoid arthritis (3%), autoimmune thrombocytopenia (3%), psoriasis (2%), ulcerative colitis (1%), and vasculitis (1%). Polymyalgia rheumatica (PMR), skin ulcers, iritis, myositis, peripheral neuropathy, Sweet’s syndrome, and pericarditis are slightly less common. Pure red cell anemia (PRCA), large granular lymphocytic leukemia (LGL) and nocturnal paroxysmal hemoglobinuria (PNH) are associated with MDS and they are also found more often than in the general population.

It is worth adding that patients with MDS, especially after the age of 65, are more likely to develop cardiovascular events (CVE), including myocardial infarction, heart failure, and arrhythmia. Within three years of MDS diagnosis, CVE have occurred in 73.2% of patients, compared to 54.5% of 1.4 million people receiving medical services for other reasons (Medicare, USA) [5].

Diagnosis
The primary examination is complete blood count (CBC) with differential (features of dyserythropoiesis, percentage of blasts). The anemia is usually macrocytic, less commonly normocytic or microcytic, and the number of reticulocytes is usually not increased. Cytopenia is a prerequisite for MDS diagnosis. In the differential diagnosis of cytopenia, it is recommended to perform basic tests, such as: iron metabolism parameters, vitamin B12 and folate acid levels, direct antiglobulin test (DAT), renal and hepatic function, and exclusion of viral diseases (hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV)).

Another examination is a bone marrow aspiration with cytomorphological, histopathological, cytochemical (Prussian blue staining to detect sideroblasts) and cytogenetic tests.

Cytomorphological bone marrow examination is decisive in assessing the percentage of blasts and the features of dysplasia, both qualitatively and quantitatively [6]. The most common dysplastic features found in patients with MDS are set out in Table I. The coexistence of cytopenia with morphological changes typical for MDS or an increased percentage of blasts allow for an MDS diagnosis. Histopathology should be routinely performed in all patients, and is particularly helpful in assessing bone marrow cellularity, fibrosis, megakaryocyte dysplasia, atypical localization of immature precursors (ALIP), and blast rates in cases where cytomorphology is not fully reliable. In 80% of patients, there is increased bone marrow (BM) cellularity, while in the remaining 20%, BM is normo- or hypocellular.

The diagnosis of MDS is also possible in patients with no obvious signs of dysplasia or increased blast percentage, if specific cytogenetic changes are present, i.e., –7 or del(7q), del(5q), and (17q) or del(17p), +12 or del(13q), del(11q), del(12p) or t(12p), del(9q), idic(X) (q13), t(11; 16) (q23; p13.3), t(3; 21) (q26.2; q22.1), t(1; 3) (p36.3; q21.1), t(2; 11) (p21; q23), inv(3) (q21q26.2), t(3; 3) (q21; q26.2), t(6; 9) (p23; q34). Cytogenetic abnormalities such as +8, del (20q) and −Y are not considered specific for MDS. Molecular and immunophenotypic tests by flow cytometry are complementary and not mandatory. MDS diagnostic criteria are set out in Table II [7].

The initial diagnostic workup for MDS is set out in Table III, while Figure 1 shows MDS diagnostic algorithm.

Differential diagnosis
The first priority in the differential diagnosis of MDS is to exclude primary extramedullary causes of cytopenia,
other hematopoietic malignancies, as well as PRCA, PNH, or bone marrow metastases. In the case of a typical family or personal medical history, especially in younger people, congenital myeloid neoplasms should be excluded. The causes leading to cytopenia/dysplasia are set out in Table IV.

Pre-MDS states

Some healthy people have somatic mutations/cytogenetic abnormalities typical for myeloid neoplasms. The lack of cytopenia and other features of MDS allows for the diagnosis of clonal hematopoiesis with indeterminate potential (CHIP). In the case of cytopenia without other features of MDS or dysplasia without cytopenia and other features of MDS, idiopathic cytopenia of undetermined significance (ICUS) and idiopathic dysplasia of undetermined significance (IDUS) are diagnosed, respectively. On the other hand, if cytopenia is revealed without a detectable cause and molecular or cytogenetic abnormalities specific for MDS, a clonal cytopenia of undetermined significance (CCUS) should be diagnosed [8].

It has not been determined whether the acquired mutations in CHIP are the result of aging or an early stage of cancer. Some patients may develop MDS in the future (0.5–1%/year). In people with CHIP, especially with mutations in epigenetic factors, an increased incidence of coexistent cardiovascular diseases has been found. The differential diagnosis of CHIP, CCUS, IDUS, and ICUS conditions is set out in Table V.

### Principles of cytogenetic testing in MDS

According to the current criteria for the diagnosis of MDS, cytogenetic testing is still one of the basic diagnostic and prognostic examinations.

Considering cytogenetic abnormalities, only the deletion of the long arm of chromosome 5 defines a distinct histoclinical entity – MDS with an isolated 5q deletion. In the current World Health Organization (WHO) classification, the scope of the concept of ‘isolated’ has been extended; this category still includes del(5q) as the only aberration, but also del(5q) with one additional aberration, other than the unfavorable chromosome 7 aberrations, e.g. del(7q) and monosomy 7 (–7) [9]. Classic cytogenetic testing in MDS should be performed before commencement of any treatment, because, for example, treatment with steroids may inhibit cell division and make it impossible to put the right diagnosis. The sample should be bone marrow, as there may be an insufficient number of CD34+ cells in the peripheral blood. The culture of the marrow cells of patients suffering from MDS is a short-term, 24- or 48-hour culture without mitogen (stimulator of cell proliferation). Chromosomes are analyzed after G-banding by trypsin with Giemsa (GTG) staining. In MDS, as a rule, at least 20 metaphases are analyzed. If it is difficult to obtain 20 metaphases, a few cells with a clonal aberration are sufficient to determine the result (clonal aberration is defined as the presence of a given trisomy or a structural aberration in at least two cells, or a given monosomy in at least three cells). On the other hand, the lack of aberration does not allow the completion of the test before the analysis of 20 cells. In about half of the karyotypes, chromosomal aberrations are found, most often –5/del (5q), –7/del(7q), +8, del(20q), –Y. The most common abnormalities are set out in Table VI [10].
The 5q deletion covering the 5q31 (EGR1) – 5q33.1 (RPS14) region and appearing alone or accompanied by a single additional aberration defined above is associated with a favorable prognosis.

Older men may experience a loss of the Y chromosome unrelated to MDS. Therefore, in this group, −Y is assigned to MDS when it occurs in more than 70% of metaphases, while in younger men the threshold is 30%. A complex karyotype (at least three independent autosomal chromosomal aberrations, i.e. autosomes) is always unfavorable. It is often accompanied by the loss of ≥1 copy of TP53 gene or its mutation. The monosomal karyotype (at least two autosomal monosomies or at least one autosomal monosomy and at least one autosomal structural aberration) is always unfavorable, and according to some authors it is worse than the complex karyotype.

If metaphasal plates are not obtained, or the number of metaphasal planes obtained, which also does not contain clonal aberrations, is suboptimal (<20), or they are unreadable, FISH with the MDS probe panel [for −5/−del(5q), −7/−del(7q), +8, del(17p)/TP53, del(20q), and possibly −Y] should be performed. Some of these probes can be used together, which speeds up FISH diagnostics. In total, 100–200 interphasic nuclei are routinely analyzed under fluorescence microscopy. It is believed that FISH detects cytogenetic abnormalities in 15% of patients with normal karyotype tested using the classical method [11].

### Immunophenotyping

The WHO classification of hematopoietic neoplasms indicates that flow cytometry (FC) is not a sufficient diagnostic method for MDS in the absence of definitive cyt morphological and/or cytogenetic criteria. However, it points out that the aberrant immunophenotypic features show a strong correlation with this diagnosis [12].

Immunophenotype abnormalities described as typical MDS are shown in 6.4% of cytopenia cases without marked dysplasia in cytological examination, however, around one third of these cases present with typical cytogenetic changes.

The European LeukemiaNet (ELN) Working Group for Flow Cytometry in MDS (IMDSFlow) proposed guidelines for the use of this method in the integrated diagnosis of MDS [13]. For screening purposes, a mini-panel (so-called Ogata score) with four parameters (one point for each) can be used [12]:

- increased CD34+ progenitors rate among all BM nucleated cells (>2%);
- reduced CD34+/CD19+ and/or CD34+ CD10+ B-cell precursors rates among all CD34+ cells;
- altered expression of CD45 on myeloblasts relative to lymphocytes (≤4 or ≥7.8);
- reduced granularity (SSC, side scatter channel) on granulocytes in relation to lymphocytes (≤6).

A total score of ≥2 points allows the diagnosis of low-risk MDS with a specificity of 92–98%. The disadvantage...
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Table III. Diagnostic tests used in myelodysplastic syndrome (MDS)

| Test                                | Material/assessment recommendations | Purpose                                                                 | Required/Recommended |
|-------------------------------------|--------------------------------------|-------------------------------------------------------------------------|----------------------|
| CBC (differential)                  | Peripheral blood                     | Cytopenia and dysplasia of one or more cell lineages                    | Required             |
| Bone marrow aspiration biopsy       | Bone marrow                          | Assessment of blasts rate                                               | Required             |
|                                    | Assessment of at least 500 cells      | Assessment of bone marrow cellularity and E:M lineage ratio             |                      |
|                                    |                                      | Assessment of blasts rate — percentage of nucleated cells regardless of dominant erythroid lineage |                      |
|                                    |                                      | Dysplasia of one or more hematopoietic cell lineages                   |                      |
| Bone marrow aspiration biopsy       | Ring-shaped sideroblasts — Prussian blue staining | Assessment of ring sideroblasts rate                                     | Required in LR-MDS   |
|                                    | Assessment of 100 erythroblasts       |                                                                         |                      |
|                                    | Biopsy ≥1 cm (formalin)              | Assessment of BM cellularity, blasts rate and dysplasia features       | Required             |
|                                    | • IHC minimal panel: CD34+, CD117/KIT, CD42b or CD61, tryptase            |                                                                         |                      |
|                                    | • Additional panel: CD3, CD14, CD20 |                                                                         |                      |
| Cytogenetic analysis               | Bone marrow (heparin) ≥5 mL          | Detection of acquired chromosomal abnormalities that may allow establishment of final diagnosis and prognosis | Required             |
| FISH                                | Blood — conditionally, in absence of BM material |                                                                         |                      |
| Flow cytometry — immunophenotyping | Bone marrow/peripheral blood         | Detection of targeted abnormalities in the case of failure of standard cytogenetic method | Recommended          |
|                                    | Bone marrow (EDTA) ≥2 mL             | Detection of abnormalities in erythroid lineage, immature cells of myeloid lineage, mature granulocytes, monocytes, immature and mature cells of lymphoid lineage | Recommended          |
| Molecular testing                  | Bone marrow (EDTA) ≥2–5 mL           | Detection of somatic mutations                                          | Recommended          |
|                                    | Skin, hair follicles                 | Detection of somatic mutations                                          | Recommended          |

BM — bone marrow; CBC — complete blood count; EDTA — ethylenediaminetetraacetic acid; FISH — fluorescence in situ hybridization; IHC — immunohistochemistry; LR-MDS — low-risk MDS

Reduction in the B-cell progenitors rate is sometimes found in the elderly, and MDS blasts may aberrantly fail to express CD34 [14].

In conclusion, flow cytometry testing in the diagnosis of MDS is useful:

- in cases of cytopenia without marked dysplasia and cytogenetic/molecular changes. The abnormalities found in FC may then indicate MDS or, in their absence, another cause of detected abnormalities;
- in MDS patients with single-lineage dysplasia in cytology examination, and with alterations of other lineages in FC. These changes may have a prognostic value or suggest incorrect classification of MDS (re-assessment of dysplasia in lineage disturbed in FC is strongly recommended).

Molecular testing in MDS

Thanks to the use of techniques based on next-generation gene sequencing, molecular disorders in the course of MDS have been found in c.80–90% of patients. Some of them have been shown to occur in MDS with a specific phenotype, but the most important observations concern the significance of the mutations for survival prognosis and transformation into AML, as well as treatment response. So
Cytopenia (1–3 lineages)
Cytopenia is defined as Hb and/or PLT and/or ANC levels below the lower limit of the laboratory norm

Medical history
- Exposure to toxic substances
- Drugs
- Medications/therapies (including CTH, RTH)
- Family history of inherited bone marrow failure (IBMF)

Complete blood count
- Automatic smear
- Microscopic smear
- Reticulocytes

Virological testing
- HBV (HBsAg), HCV (anti-HCV), HIV, EBV, CMV

Biochemistry
- Vitamin B12, folic acid
- Iron metabolism (Fe, TIBC, ferritin, TSAT)
- Liver function (transaminases, bilirubin)
- ANA and anti-dsDNA
- Cu
- Ceruloplasmin
- DAT

Imaging studies
- Abdominal ultrasound
- Coagulogram (APTT, INR, fibrinogen)

Drugs
- Exposure to toxic substances
- Medications/therapies (including CTH, RTH)

Other causes, e.g.: infiltration of another neoplasm or other cells, hypersplenism, Gaucher disease

Bone marrow biopsy
- Marrow aspirate + sideroblasts staining
- Histopathology (trepanobiopsy)
- FACS
- Cytogenetic research

Dysplasia ≥10% of cells of a given line and/or blasts 5–19% and/or specific cytogenetic abnormalities and/or RS ≥15%

ICUS (CCUS)

No

The presence of additional features:
- Monocytosis >1 × 10⁹/L
- Hypocellular bone marrow (cellularity <30%)
- Myelofibrosis
- Suspicion of germ cells neoplasm

No

Monocytosis >1 × 10⁹/L
- CMML

Hypocellular bone marrow (cellularity <30%)
- Hypoplasia/AA/PRCA
- T-LGL
- PNH

Myelofibrosis
- Molecular studies: JAK2, CALR, MPL
- PMF

Suspected germ cell neoplasm — molecular test (NGS)

No

Probable MDS
No

More likely cause other than MDS
Figure 1. Diagnostic algorithm of myelodysplastic syndromes (MDS); *if mutation SF3B1 (splicing factor 3b subunit 1) is present; AA — aplastic anemia; ANA — anti-nuclear antibodies; ANC — absolute neutrophil count; anti-dsDNA — anti-double stranded DNA; anti-HCV — anti-hepatitis C virus test; APTT — activated partial thromboplastin time; BM — bone marrow; CCUS — clonal cytopenia of undetermined significance; CTH — chemotherapy; CMML — chronic myelomonocytic leukemia; CMV — cytomegalovirus; CRP — C-reactive protein; EBV — Epstein-Barr virus; EPO — erythropoietin; ESR — erythrocyte sedimentation rate; FACS — fluorescence-activated cell sorting; Hb — hemoglobin; HBsAg — hepatitis B surface antigen; HBV — hepatitis B virus; HCV — hepatitis C virus; HIV — human immunodeficiency virus; ICUS — idiopathic cytopenia of undetermined significance; INR — international normalized ratio; LDH — lactate dehydrogenase; MDS-del 5 — multilineage dysplasia; NGS — next-generation sequencing; PB — peripheral blood; PLT — platelets; PMF — primary myelofibrosis; PNH — paroxysmal nocturnal hemoglobinuria; PRCA — pure red cell leukemia; RS — ring sideroblasts; RTH — radiotherapy; SLD — single lineage dysplasia; TIBC — total iron-binding capacity; T-LGL — T-cell large granular lymphocytic leukemia; TSAT — transferrin saturation; U — unclassified.
### Table IV. Differential diagnosis of cytopenia/dysplasia

| Diagnosis                      | Bone marrow/morphology                                                                 | Other tests (serum)                                                                                                                                 |
|--------------------------------|----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|
| Vitamin $B_{12}$ deficiency    | Megaloid forms of erythroid and granulocytic lineages, irregular shapes of erythroblast nuclei, Howell-Jolly bodies, neutrophil nucleus hypersegmentation, macrocytosis, pancytopenia, decreased number of reticulocytes, hypercellular cell bone marrow | ↓ vitamin $B_{12}$ ↓ holotranscobalamin ↑ methylmalonic acid (MMA) ↓ homocysteine ↓ LDH ↓ bilirubin ↓ folic acid ↓ homocysteine MMA in normal range ↓ cholesterol ↑ ALT ↓ Mg, Zn, P, K, Na ↓ estrogen, testosterone ↓ FT3, FT4 ↓ Cu ↓ Pb, Hg, Cd, As ↓ serum Fe ↑ GGTP |
| Folic acid deficiency         | Similar to vitamin $B_{12}$ deficiency                                                  |                                                                                                                                                   |
| Eating syndromes              | Anorexia                                                                               | Hypocellular bone marrow, possible ‘gelatinous transformation’ of bone marrow and/or cell necrosis, more numerous histiocytes, peripheral blood acanthocytes, anemia, leukopenia, less often thrombocytopenia |
|                                | Copper deficiency                                                                      | Pancytopenia, vacuolization of cytoplasm of erythroid and granulocytic lineages, ring-shaped sideroblasts                                        |
| Exposure to heavy metals (including lead Pb, Hg, Cd, As) | Hypocellular bone marrow, ring-shaped sideroblasts, pancytopenia, basophilic spotting |                                                                                                                                                   |
| Alcohol abuse                 | Vacuolization of cytoplasm of erythroid and granulocytic lineages, ring-shaped sideroblasts, hyperplasia of erythroid lineage, hypocellular bone marrow, macrocytosis, pancytopenia, symptoms resolve after alcohol withdrawal; stomatocytes, acanthocytes are present in liver cirrhosis |                                                                                                                                                   |
| Cytostatics                   | Most cytostatics                                                                       | After higher doses — hypoplasia  
Regeneration of megakaryocytic and erythroid lineages usually precedes regeneration of granulocytic lineage  
After low doses — megaloblastic regeneration, dyserythropoiesis  
Macrocytosis  
Bone marrow hypoplasia, dysplasia of granulocytic and megakaryocytic lineages, mono-, duo-, pancytopenia  
Sideroblastic anemia  
Hypersegmentation of neutrophil nuclei, dyserythropoiesis  
Granulocytic dysplasia with high doses of corticosteroids (resolves 1–4 weeks after discontinuation)  
↑ neutrophils, ↓ lymphocytes  
Granulocyte lineage: toxic granules, ‘left shift’, vacuolization of cytoplasm, abnormal lobulization of neutrophil nuclei, ↑ neutrophils |
| Anticonvulsants, antithyroid drugs, antibiotics | Hydroxyurea, cyclophosphamide, methotrexate, azathioprine |                                                                                                                                                   |
| Isoniazid, linezolid           |                                                                                       |                                                                                                                                                   |
| Trimethoprim/sulfamethoxazole, tacrolimus, mycophenolate mofetil, azathioprine |                                                                                       |                                                                                                                                                   |
| Steroids                      |                                                                                       |                                                                                                                                                   |
| G-CSF                         |                                                                                       |                                                                                                                                                   |
### Table IV (cont.). Differential diagnosis of cytopenia/dysplasia

| Diagnosis                                      | Bone marrow/morphology                                                                 | Other tests (serum)                                                                 |
|-----------------------------------------------|----------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Infections                                    |                                                                                        |                                                                                     |
| HIV                                           | Dysplasia (especially aggravated during antiretroviral therapy), megaloblastic reaction, cytopenia, macrocytosis | Serological tests, PCR                                                               |
| Parvovirus B19                                 | Hypoplasia of erythroid lineage, presence of single, giant basophils                     | PCR — parvovirus B19                                                                 |
| HBV, HCV                                      | Lymphocyte clusters, reactive plasmocytes, dyserythropoiesis, cytopenia (more often thrombocytopenia and anemia) | ↑ bilirubin                                                                          |
| EBV, CMV                                      | Possible cytopenias, monocytoid cells, activated lymphocytes, possible development of HLH, NHL | ↑ AST, ALT, ALP, INR, Serological tests, PCR                                         |
| Leishmaniasis                                 | Pancytopenia, dysplasia, fibrosis, hemophagocytosis, iron deposits                       | Serological tests, PCR                                                               |
| Bacterial                                     | Neutrophils: toxic granules, Doehle bodies                                              | ↑ CRP, culture                                                                       |
| Autoimmune                                    |                                                                                        |                                                                                     |
| Autoimmune hemolytic anemia (AIHA)            | Anemia, slight erythroid lineage dysplasia                                             | Antinuclear antibodies, rheumatoid factor                                             |
| Primary immune thrombocytopenia (ITP)         | Possible dysplasia of megakaryocytic lineage (micromegakaryocytes)                     | Positive DAT, ↑ bilirubin, ↑ LDH, ↓ haptoglobin                                        |
| Autoimmune neutropenia (including Felty’s syndrome) | Neutropenia, possible dysplasia, possible T-cell clone                                 | Anti-neutrophilic antibodies, splenomegaly, rheumatoid factor                         |
| Hypothyroidism (Hashimoto’s disease)          | Possibility of erythroid lineage hypoplasia, anemia, macrocytosis, ↓ reticulocytes     | TSH, FT3, FT4, anti-TG antibodies, anti-TPO antibodies                                |
| Chronic idiopathic neutropenia               | Normal bone marrow or hypoplasia of granulocytic lineage, possible lymphopenia         |                                                                                     |
| Post-transplant                               |                                                                                        |                                                                                     |
| Liver                                         | Possible trilinear dysplasia, macrocytosis of erythrocytes                              |                                                                                     |
| allo-HCT                                      | Hypoplastic bone marrow, dyserythropoiesis, risk of t-MDS/t-AML                         |                                                                                     |
| Aplastic anemia                               | Hypocellular bone marrow, mainly lymphocytes present, anemia, duo- or pancytopenia, ↓ reticulocytes | ↑ ferritin, TG, ↓ fibrinogen, ↑ sCD25                                              |
| HLH                                           | Cytopenia, hemophagocytosis and to a lesser extent dysplasia                            | Imaging tests — splenomegaly                                                         |
| PNH                                           | Cytopenia, hypo-/normocellular bone marrow                                            | FACS: ↓ FLAER, CD 55, CD59 on granulocytes, monocytes and erythrocytes, ↑ bilirubin   |
| LGL                                           | Neutropenia, pancytopenia, possibility of hypoplastic bone marrow, infiltration of clonal T or NK lymphocytes in bone marrow, granular lymphocytes in peripheral blood | Possibility of a positive rheumatoid factor, imaging tests: splenomegaly             |
| Congenital bone marrow failure syndromes (e.g. Fanconi anemia) | Cytopenias                                                                             | Typical genetic abnormalities                                                        |
### Table IV (cont.). Differential diagnosis of cytopenia/dysplasia

| Diagnosis | Bone marrow/morphology | Other tests (serum) |
|-----------|------------------------|---------------------|
| Other hematopoietic malignancies (aCML, PMF, CMML) | 1–3 linear dysplasia, fibrosis, anemia, thrombocytopenia Monocytosis >1 × 10⁹/L (CMML) or immature forms of granulocyte lineage >10% peripheral blood WBC (aCML) or erythroblasts (PMF) | JAK2 V617F, MPL, CALR genes mutations imaging tests — spleno- and/or hepatomegaly |
| Congenital sideroblastic anemia (e.g. XLSA) | Erythroid lineage dysplasia, ring-shaped sideroblasts, Pappenheimer bodies, ↓ MCV | Typical genetic abnormalities (e.g. ALAS2 gene mutation) ↓ ferritin, hepatosplenomegaly |

| J — reduced value; ↑ — increased value; aCML — atypical chronic myeloid leukemia; ALAS2 — aminolaevulinic acid synthetase 2; allo-HCT — allogeneic hematopoietic cell transplantation; anti-TG — anti-thyro-globulin antibodies; ALP — alkaline phosphatase; ALT — alanine aminotransferase; anti-TPO — anti-thyreoperoxidase antibodies; As — arsenic; AST — aspartate aminotransferase; Cd — cadmium; CMML — chronic myelomonocytic leukemia; Cu — cuprum; DAT — direct antiglobulin test; ESR — erythrocyte sedimentation rate; FACS — flow cytometric immunophenotypic testing; Fe — ferrum; FLAER — alexa 488 proaerolysin variant; FT3 — free triiodothyronine; FT4 — free thyroxine; G-CSF — granulocyte colony-stimulating factor; GGTP — gamma-glutamyltranspeptidase; Hg — mercury; HIV — human immunodeficiency virus; HLH — hemophagocytic lymphohistiocytosis; INR — international normalized ratio; ITP — primary immune thrombocytopenia; K — potassium; LDH — lactate dehydrogenase; LGL — large granular lymphocytic leukemia; line E — erythroid lineage; line G — granulocytic lineage; line M — megakaryocytic lineage; MCV — mean corpuscular volume; Mg — magnesium; MMA — methylmalonic acid; MPV — mean platelet volume; Na — sodium; NAIH — autoimmune hemolytic anemia; P — phosphorus; Pb — plumbum; PCR — polymerase chain reaction; PDW — platelet distribution width; PMF — primary myelofibrosis; PNH — paroxysmal nocturnal hemoglobinuria; RA — rheumatoid arthritis; sCD25 — soluble interleukin 2 receptor; SLE — systemic lupus erythematosus; TSH — thyroid-stimulating hormone; Zn — zinc |

### Table V. Pre-myelodysplastic syndrome (MDS) conditions

| Idiopathic cytopenia of undetermined significance (ICUS) | Idiopathic dysplasia of undetermined significance (IDUS) | 
|---------------------------------------------------------|----------------------------------|
| **Cytopenia** (s) in CBC* No apparent causes of cytopenia Failure to meet criteria for MDS diagnosis | No cytopenia in CBC Failure to meet criteria for MDS diagnosis | ICUS + IDUS — pre-MDS conditions without genetic abnormalities typical of MDS |
| No genetic abnormalities typical for MDS** Absence or presence of dysplasia in <10% of cells of a given hematopoietic lineage <5% blasts*** | No genetic abnormalities typical of MDS** Dysplasia features in ≥10% of cells of a given hematopoietic lineage <5% blasts*** | |
| **Clonal cytopenia of undetermined significance (CCUS) | Clonal hemopoiesis of indeterminate potential (CHIP) |
| **Cytopenia** (s) in CBC* No apparent causes of cytopenia Failure to meet criteria for MDS diagnosis | No cytopenia in CBC Failure to meet criteria for MDS diagnosis | CCUS + CHIP — pre-MDS conditions with genetic abnormalities typical of MDS |
| Genetic abnormalities typical for MDS (one or more)** Absence or presence of dysplasia in <10% of cells of a given hematopoietic lineage <5% blasts*** | Genetic abnormalities typical for MDS (one or more)** Dysplasia features in <10% of cells of a given hematopoietic lineage <5% blasts*** | |
| ICUS + CCUS — pre-MDS conditions with cytopenias | IDUS + CHIP — pre-MDS conditions without cytopenias |

*Any grade cytopenia lasting more than four months after excluding other causes; ** abnormalities identified by G-banding by trypsin with Giemsa (GTC) cytogenetics, fluorescence in situ hybridization (FISH) or by molecular biology. In the case of sequencing methods, a variant allele frequency (VAF) of at least 2% is considered to be diagnostic for pre-MDS conditions; for diagnosis of MDS, VAF should be ≥10%; *** blasts found in peripheral blood and/or bone marrow smears; CBC — complete blood count.
far, the occurrence of sequence variants has been demonstrated in as many as 181 genes, but only in 79 of them was it repeatable [15]. Two of the largest studies, involving a total of 1,682 patients, tested 104–111 genes and significant mutations were found in 43–47 genes [16, 17].

The most common mutations in MDS are detected in genes responsible for the following functions:

- **pre-mRNA splicing.** Sequence variants in SF3B1 (splicing factor 3b subunit 1), SRSF2 (serine and arginine rich splicing factor 2), U2AF1 (U2 small nuclear RNA auxiliary factor 1) and ZRSR2 (zinc finger CCCH-type, RNA binding motif and serine/arginine rich 2) genes are among the most frequently reported disorders regulating splicing in MDS. They are found in almost 70% of patients, and are the most specific for MDS. SF3B1 is the only gene whose sequence variants are included in the 2016 WHO revised classification, as well as in the 2020 proposal for a new MDS subtype with the SF3B1 mutation [18]. The sequence variants in SF3B1 gene are closely related to the presence of ring sideroblasts and is helpful in defining the myelodysplastic syndrome with ring sideroblasts (MDS-RS) subtype classification. Alterations of SF3B1 gene occur proportionally more frequently in lower-risk patients according to the Revised International Prognostic Scoring System (IPSS-R). Sequence variants in SRSF2 gene are indicative of an unfavorable course of MDS, with an increased risk of leukemic transformation;

- **epigenetic DNA modification.** Sequence variants in TET2 (ten eleven translocation-2) gene are found quite frequently, in 19–25% of MDS cases, but are also present in other myeloid neoplasms. The presence of mutation predicts a good response to azacytidine, which, however, is not associated with an increase in survival time. Sequence variants in the DNMT3A (DNA-methyl transferase 3 alpha) gene occur mainly in elderly patients with a frequency of 3–8% in MDS. They are found in all of the marrow cells. The presence of mutation is associated with shorter survival;

- **chromatin modification.** Sequence variants in ASXL1 (associated sex combs-like 1) gene are among the more frequent molecular changes in MDS, occurring in 15–20% of patients. Their presence in MDS adversely affects overall survival and is associated with a higher probability of transformation into AML. Sequence variants in EZH2 (enhancer of zeste homolog 2) gene occur in 5–10% of myelodysplastic syndromes and are a marker of a poor prognosis;

- **DNA repair (ATM, DLRE1C, FANCL, BRCC3),** as well as cohesive complex (CTCF, RAD21, STAG2, SMAC1A);

- **RAS signaling pathway (KRAS, NRAS, CBL, NF1, PTPN11);**

- **transcription factors (RUNX1, GATA, TP53, CEBPA, BCOR, NCOA, PHF6, ET6).** Sequence variants in TP53 gene (tumor protein 53) occur in 5–14% of myelodysplastic syndromes and are a marker of a poor prognosis.

### Table VI. Commonest cytogenetic abnormalities in myelodysplastic syndrome patients (acc. to Schanz et al. [10])

| Cytogenetic abnormalities | Incidence [%] |
|---------------------------|---------------|
| Single abnormalities:     |               |
| • del(5q)                 | 6.4           |
| • +8                      | 4.7           |
| • -Y                      | 2.2           |
| • del(20q)                | 1.7           |
| • -7                      | 1.6           |
| • del(11q)                | 0.7           |
| • del(12p)                | 0.6           |
| • del(7q)                 | 0.5           |
| • i(17q)                  | 0.4           |
| • +19                     | 0.4           |
| • inv(3)/t(3q)/del(3q)    | 0.4           |
| • +21                     | 0.3           |
| • der(1;7)                | 0.3           |
| • –13/del(13q)            | 0.3           |
| • –21                     | 0.3           |
| • –X                      | 0.3           |
| • Other single            | 5.8           |
| Double abnormalities:     |               |
| • including del(5q)       | 1.6           |
| • including -7/del(7q)    | 1.2           |
| • including other abnormalities | 3.4       |
| Complex abnormalities:    |               |
| • 3 aberrations           | 2.1           |
| • >3 aberrations          | 7.0           |
| Independent clones        | 0.9           |
| Clonal evolution          | 13.4          |
signal transduction factors (JAK2, FLT3, KIT, MLP, GNAS). In more than 10% of patients, sequence variants are found in six genes: TET2, SF3B1, ASXL1, SRSF2, DNMT3A and RUNX1, in 8–12% of patients in two genes U2AF1 and TP53, and in 5–10% of patients in four genes: EZH2, ZRSR2, STAG2, NRAS.

WHO classification 2016, 2020

In the current modification of the 2016 classification, all types of syndromes are preceded by the name MDS [9] (see Table VII). Specific subtype without excess blasts and 5q deletion is primarily determined by the number of dysplastic cell lineages, and not by cytopenia.

Based on the data of 3,479 patients, of whom 795 had SF3B1 gene mutation, an additional group of experts of the International Working Group for the Prognosis of MDS (IWG PM) proposed in 2020 to distinguish a new MDS subtype, i.e. MDS with SF3B1 mutation (Table VIII). The authors showed that the presence of SF3B1 gene mutation without ring sideroblasts (RS) had significant prognostic value. Patients with refractory anemia with ring sideroblasts (RARS) or refractory cytopenia with multilineage dysplasia (RCMD-RS) subtype with the SF3B1 mutation live statistically significantly longer than patients with RARS or RCMD without SF3B1 mutation. The effect of SF3B1 mutation on survival is significant only in the group with blasts rate <5% in BM and <1% in peripheral blood without deletion 5q, monosomy 7 and chromosome 3 disorders, as well as in patients without EZH2 and RUNX1 mutations [18].

Table VII. 2016 World Health Organization (WHO) classification of myelodysplastic syndromes (source [9])

| Entity | Number of dysplastic lineages | Cytopenias* | Ring sideroblasts (%) | Blasts in bone marrow (BM) and peripheral blood (PB) [%] |
|--------|-------------------------------|-------------|-----------------------|-------------------------------------------------------|
| MDS with single lineage dysplasia, MDS-SLD | 1 | 1 or 2 | <15/<5** | BM <5, PB <1, without Auer rods |
| MDS with multilineage dysplasia, MDS-MLD | 2 or 3 | 1-3 | <15/<5** | BM <5, PB <1, without Auer rods |
| MDS with ring sideroblasts (MDS-R) | MDS-R-SLD | 1 | 1 or 2 | ≥15/≥5** | BM <5, PB <1, without Auer rods |
| | MDS-R-MLD | 2 or 3 | 1-3 | ≥15/≥5** | BM <5, PB <1, without Auer rods |
| MDS with isolated del(5q)*** | 1-3 | 1-2 | None or any | BM <5, PB <1, without Auer rods |
| MDS with excess blasts-MDS-EB | MDS-EB1 | 0-3 | 1-3 | None or any | BM <5 or PB 2-4, without Auer rods |
| | MDS-EB2 | 0-3 | 1-3 | None or any | BM 10-19 |
| MDS unclassifiable, MDS-U | With 1% blasts in PB | 1-3 | 1-3 | None or any | BM <5, PB = 1 without Auer rods |
| | Pancytopenia with unilineage dysplasia | 1 | 3 | None or any | BM <5, PB <1, without Auer rods |
| | Without dysplasia with a cytogenetic alteration defining MDS**** | 0 | 1-3 | None or any | BM <5, PB <1, without Auer rods |
| | Del (5q) and pancytopenia | 1-3 | 3 | None or any | BM <5, PB <1, without Auer rods |

*Cytopenias are defined as follows: hemoglobin (Hb) <10.0 g/dL, platelet count <100 × 10^9/L, absolute neutrophil count <1.8 × 10^9/L. Myelodysplastic syndrome (MDS) can rarely present with mild anemia or thrombocytopenia above the indicated values. Peripheral blood monocytes <1 × 10^9/L; **presence of SF3B1 gene mutation; ***presence of cytogenetic del (5) abnormalities ± additional aberration without chromosome 7 abnormalities; ****presence of typical cytogenetic abnormalities; BM – bone marrow; PB – peripheral blood

Congenital myeloid neoplasms

Myeloid neoplasms in which the predisposing genetic defect is inherited or arises de novo at an early stage of embryogenesis and is present in all (including terminal) cells in the body are known as myeloid neoplasms with germline predisposition (MNGP) or hereditary myeloid malignancy syndromes (HMMSs). Most often they concern genes such as: CEBPA, DDX41, RUNX1, ANKRD26, ETV6, GATA2, SRP72, and SAMD9. The exact frequency of these diseases is unknown, but it is estimated that they affect 1–5% of myeloid neoplasms. MNGP/HMMS can occur in as isolated abnormality or with concomitant diseases (including other neoplasms — lymphoid or solid tumors) or as part of complex syndromes [20–22]. The possibility of MNGP/HMMS should always be considered in patients with MDS and the following features:
Table VIII. Myelodysplastic syndromes (MDS) with SF3B1 gene mutation: International Working Group (IWG) 2020 diagnostic criteria (source [18])

| Criteria                                                                 | Definition                                                                 |
|--------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Peripheral blood cytopenia defined as reduction of parameters below laboratory standards in at least one cell lineage: erythroid, neutrophil and platelets |
| Presence of somatic mutation of SF3B1 gene                              |
| Isolated erythroid or multilineage dysplasia*                            |
| Blasts in bone marrow <5% and in peripheral blood <1%                    |
| Failure to meet criteria for diagnosis of other diseases: MDS with isolated 5q deletion, MDS/MPN-RS-T and other MDS/MPN as well as primary myelofibrosis and other myeloproliferative neoplasms according to WHO 2016 classification |
| Normal karyotype or cytogenetic abnormalities with exception of: del(5q), chromosome 7 monosomy, inv(3) or 3q26 abnormalities, composite karyotype (≥3 abnormalities) |
| Coexistence of other somatic mutations except RUNX1 and/or EZH2**         |

*Presence of ring sideroblast is not required; **presence of JAK2/VEG1F, CALR or MPL mutation strongly suggests MDS/MPN-RS-T (a myelodysplastic/myeloproliferative neoplasm with an excess of ring sideroblasts and thrombocytosis); WHO — World Health Organization

- clinical symptoms or other diseases suggesting one of the congenital syndromes [20];
- ≥2 cases of MDS/AML in a family;
- ≥2 cases of aplastic anemia/unexplained cytopenia in a family;
- congenital syndrome diagnosed in relative;
- pathogenic mutation in the gene associated with congenital myeloid neoplasms, in particular:
  - CEBPA biallelic mutation (10% of cases have a germline mutation),
  - GATA2 mutation and/or chromosome 7 monosomy in a very young individual (most cases affect children and adolescents),
  - RUNX1 mutation,
  - DDX41 mutation;
- patients with and/or family history of multiple cancers (with the exception of chronic lymphocytic leukemia);
- related bone marrow donor of a patient with diagnosed congenital syndrome.

In addition, the possible presence of MNGP/HMMS should always be considered if:
- unexplained cytopenias are found;
- there are difficulties with hematopoietic cells mobilization.

Testing procedure for suspected MNGP

Testing of germline DNA from fibroblast cultures obtained by skin biopsy is the gold standard for MNGP/HMMS diagnostics. Other sources of DNA used include:
- skin cells examined immediately after skin specimen collection (no culture);
- hair follicles, nails (small amount of material);
- buccal swab/saliva (material quick and easy to collect, but possible contamination with blood and cancer cells).

Germinal DNA testing should also be performed in relatives and potential related donors of hematopoietic cells. In this situation, fibroblast culture is also the gold standard.

Prognostic factors

Prognostic and predictive scores for MDS patients have been published, including responses to immunosuppressive therapy, responses to treatment with erythropoiesis stimulating proteins or intensive chemotherapy. The most important is the International Prognostic Scoring System (IPSS) published in 1997 by Greenberg et al. and its modified 2012 version known as IPSS-R (see Table IX) [23, 24]. The International Prognostic Scoring System (IPSS) index stratifies patients depending on BM blasts percentage, cytogenetic abnormalities, and the number of peripheral blood cytopenia. IPSS risk stratification correlates with overall survival and transformation time to acute myeloid leukemia. The IPSS index is of significant clinical importance as it helps to qualify patients for a given type of treatment. The modified IPSS-R International Scoring System based on the analysis of data from 7,012 patients introduces five cytogenetic groups (with 18 subgroups of karyotype variants) and new, more detailed ranges for BM blasts rate, hemoglobin concentration, and the number of platelets and neutrophils. A limitation of the discussed indices is the lack of consideration of patient-related factors such as age, general condition and comorbidities. These factors are particularly important in qualifying for allotransplantation of hematopoietic cells. Della Porta et al. [25] have developed an index of comorbidities specific for MDS patients.

In 2005, Malcovati et al. [26] demonstrated that the dependence on red blood cell (RBC) transfusion assessed at any time point during the disease (defined as the necessity to transfuse ≥1 RBC within 8 weeks) is an important factor associated with shorter survival. Adverse prognostic factors in patients with MDS also include: increased ferritin and LDH and hypoalbuminemia. In recent years, much attention has been paid to molecular studies in MDS patients, which have been discussed in detail above. Bejar et al. [27] showed that the presence of a mutation of at least one of the following genes: TP53, EZH2, ETV6, RUNX1 or ASXL1 causes the patient to move to the next prognostically less favorable group according to IPSS. This is of particular importance in low-risk patients according to IPSS, as it allows to qualify them earlier for more aggressive treatment [27]. It seems that the implementation of molecular test results in MDS prognostic indices is an inevitable process, and additional research is still ongoing.
Table IX. Prognostic indices used in patients with myelodysplastic syndromes (sources [23, 24])

### International Prognostic Scoring System (IPSS)

| Score | 0 | 0.5 | 1.0 | 1.5 | 2.0 |
|-------|---|-----|-----|-----|-----|
| Blasts in BM (%) | <5 | 5–10 | – | 11–20 | 21–30 |
| Karyotype | Normal: –Y del(5q) del(20q) | Other abnormalities | Complex (≥3 abnormalities) chromosome 7 aberrations | – | – |
| Cytopenias | 0–1 | 2–3 | – | – | – |

**Risk groups**

| | Low | Intermediate-1 | Intermediate-2 | High |
|---|-----|----------------|----------------|------|
| 0 points | 0.5–1 points | 1.5–2 points | ≥2.5 points |

**Median overall survival**

| | Low risk | Intermediate-1 | Intermediate-2 | High |
|---|---------|----------------|----------------|------|
| 5.7 years | 3.5 years | 1.2 years | 0.4 years |

**Mean time to transformation into AML (25% of the group)**

| | Low risk | Intermediate-1 | Intermediate-2 | High |
|---|---------|----------------|----------------|------|
| 9.4 years | 3.3 years | 1.1 years | 0.2 years |

### Revised International Prognostic Scoring System (IPSS-R)

| Score | 0 | 0.5 | 1 | 1.5 | 2 | 3 | 4 |
|-------|---|-----|---|-----|---|---|---|
| Karyotype | –Y del(11q) | Normal del(5q) del(12p) del(20q) 2 abnormalities including del(5q) | del(7q) +8 +19 i(17q) others (1 abnormality) others (2 abnormalities) | –7 inv(3) t(3q) del(3q) 2 abnormalities (including –7/del(7q) 3 abnormalities) | >3 abnormalities |
| Blasts in BM (%) | ≤2 | >2–<5 | 5–10 | >10 |
| Hemoglobin [g/dL] | ≥10 | 8–<10 | <8 |
| Platelets [× 10⁹/L] | ≥100 | 50–<100 | <50 |
| Neutrophils [× 10⁹/L] | ≥0.8 | <0.8 |

**Risk groups**

| | Very low | Low | Intermediate | High | Very high |
|---|----------|-----|--------------|------|-----------|
| ≤1.5 points | >1.5–3.0 points | >3.0–4.5 points | >4.5–6.0 points | >6.0 points |

**Proportion of patients at specified risk**

| | Very low | Low | Intermediate | High | Very high |
|---|----------|-----|--------------|------|-----------|
| 19% | 38% | 20% | 13% | 10% |
Table IX (cont.). Prognostic indices used in patients with myelodysplastic syndromes (sources [23, 24])

| Revised International Prognostic Scoring System (IPSS-R) | Median overall survival | Mean time to transformation into AML (25% of group) |
|----------------------------------------------------------|-------------------------|---------------------------------|
| **Very low risk** | Low risk | Indirect risk | High risk | Very high risk |
| 8.8 years | 5.3 years | 3 years | 1.6 years | 0.8 years |
| **Mean time to transformation into AML (25% of group)** | | | |
| Very low risk | Low risk | Intermediate risk | High risk | Very high risk |
| NR | 10.8 years | 3.2 years | 1.4 years | 0.73 years |

*Hemoglobin (Hb) <10 g/dL; neutrophils <1,8 × 10⁹/L; platelets <100 × 10⁹/L; AML — acute myeloid leukemia; BM — bone marrow; IPSS — International Prognostic Scoring System; NR — not reached

Authors’ contributions
Conception and design: KM, JDT. Manuscript writing, final approval of the manuscript: all authors.

Conflict of interest
None.

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Ethics
The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; uniform requirements for manuscripts submitted to Biomedical journals.

References
1. Małdy K, Machowicz R, Waszczuk-Gajda A, et al. Demographic, hematologic, and clinical features of myelodysplastic syndrome patients: results from the First Polish Myelodysplastic Syndrome Registry. Acta Haematol. 2015; 134(2): 125–134, doi: 10.1159/000375149, indexed in Pubmed: 25925777.

2. Drozd-Sokołowska JE, Małdy K, Waszczuk-Gajda A, et al. Are myelodysplastic syndromes underdiagnosed in Poland? A report by the Polish Adult Leukaemia Group. Eur J Haematol. 2017; 98(2): 154–159, doi: 10.1111/ejh.12814, indexed in Pubmed: 27899872.

3. Ma X, Does M, Raza A, et al. Myelodysplastic syndromes: incidence and survival in the United States. Cancer. 2007; 109(8): 1536–1542, doi: 10.1002/cncr.22570, indexed in Pubmed: 17345612.

4. Komoroski RS, Kulasekararaj A, Al Ali NH, et al. Autoimmune diseases and myelodysplastic syndromes. Am J Hematol. 2016; 91(5): E280–E283, doi: 10.1002/ajh.24333, indexed in Pubmed: 26875020.

5. Goldberg SL, Chen ER, Corral M, et al. Incidence and clinical complications of myelodysplastic syndromes among United States Medicare beneficiaries. J Clin Oncol. 2010; 28(17): 2847–2852, doi: 10.1200/JCO.2009.25.2395, indexed in Pubmed: 20421543.

6. Delia Porta MG, Travaglino E, Boveri E, et al. Rete Ematologica Lombarda (REL) Clinical Network. Minimal morphological criteria for defining bone marrow dysplasia: a basis for clinical implementation of WHO classification of myelodysplastic syndromes. Leukemia. 2015; 29(1): 66–75, doi: 10.1038/leu.2014.161, indexed in Pubmed: 24935723.

7. Valent P, Orazi A, Steensma DP, et al. Proposed minimal diagnostic criteria for myelodysplastic syndromes (MDS) and potential pre-MDS conditions. Oncotarget. 2017; 8(43): 73483–73500, doi: 10.18632/oncotarget.19008, indexed in Pubmed: 29088721.

8. Valent P. ICUS, IDUS, CHIP and CCUS: diagnostic criteria, separation from MDS and clinical implications. Pathobiology. 2019; 86(1): 30–38, doi: 10.1159/000489042, indexed in Pubmed: 29860246.

9. Hasserjian R, Orazi A, Bruning R, et al. Myelodysplastic syndromes: overview. In: Swerdlow S, Campo E, Harris N, et al. ed. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, revised 4th ed. Vol. 2. International Agency for Research on Cancer, Lyon 2017: 98–106.

10. Schanz J, Tüchler H, Solé F, et al. New comprehensive cytogenetic scoring system for primary myelodysplastic syndromes (MDS) and oligoblastic acute myeloid leukemia after MDS derived from an international database merge. J Clin Oncol. 2012; 30(8): 820–829, doi: 10.1200/JCO.2011.35.6394, indexed in Pubmed: 22331955.

11. Cherry AM, Brockman SR, Paternoster SF, et al. Comparison of interphase FISH and metaphase cytogenetics to study myelodysplastic syndrome: an Eastern Cooperative Oncology Group (ECOG) study. Leuk Res. 2003; 27(12): 1085–1090, doi: 10.1016/s0145-2126(03)00104-8, indexed in Pubmed: 12921944.

12. Ogata K, Della Porta MG, Malcovati L, et al. Diagnostic utility of flow cytometry in low-grade myelodysplastic syndromes: a prospective validation study. Haematologica. 2009; 94(8): 1086–1090, doi: 10.3324/haematol.2009.086532, indexed in Pubmed: 19546439.

13. Porwit A, van de Loosdrecht AA, Bettelheim P, et al. Revisiting guidelines for integration of flow cytometry results in the WHO classification of myelodysplastic syndromes-proposal from the International/European LeukemiaNet Working Group for Flow Cytometry in MDS. Leukemia. 2014; 28(9): 1793–1798, doi: 10.1038/leu.2014.191, indexed in Pubmed: 24919805.

14. van de Loosdrecht AA, Westers TM. Cutting edge: flow cytometry in myelodysplastic syndromes. J Natl Compr Canc Netw. 2013; 11(7): 892–902, doi: 10.6004/jnccn.2013.0106, indexed in Pubmed: 23847222.
15. Zheng G, Chen P, Palavajjalla A, et al. The diagnostic utility of targeted gene panel sequencing in discriminating etiologies of cytopenia. Am J Hematol. 2019; 94(10): 1141–1148, doi: 10.1002/ajh.25592, indexed in Pubmed: 31350794.

16. Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2013; 122(22): 3616–3627, doi: 10.1182/blood-2013-08-518886.

17. Haferlach T, Nagata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia. 2014; 28(2): 241–247, doi: 10.1038/leu.2013.336, indexed in Pubmed: 24220272.

18. Malcovati L, Stevenson K, Papaemmanuil E, et al. SF3B1-mutant MDS as a distinct disease subtype: a proposal from the International Working Group for the Prognosis of MDS. Blood. 2020; 136(2): 157–170, doi: 10.1182/blood.2020004850.

19. Bernard E, Nannya Y, Hasserjian RP, et al. Implications of TP53 allelic state for genome stability, clinical presentation and outcomes in myelodysplastic syndromes. Nat Med. 2020; 26(10): 1549–1556, doi: 10.1038/s41591-020-1008-z, indexed in Pubmed: 32747829.

20. University of Chicago Hematopoietic Malignancies Cancer Risk Team. How I diagnose and manage individuals at risk for inherited myeloid malignancies. Nat Med. 2020; 26(10): 1549–1556, doi: 10.1038/s41591-020-1008-z, indexed in Pubmed: 32747829.

21. Kraft IL, Godley LA, Kraft IL, et al. Identifying potential germline variants from sequencing hematopoietic malignancies. Blood. 2020; 136(22): 2498–2506, doi: 10.1182/blood.2020006910, indexed in Pubmed: 33236764.

22. Peterson L, Bloomfield C, Niermeyer C, Godley L, et al. Myeloid neoplasms with germline redosposition. In: Swerdlow S, Campo E, Harris N, et al. ed. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, revised 4th ed. Vol. 2. International Agency for Research on Cancer, Lyon 2017: 121–128.

23. Greenberg P, Cox C, LeBeau MM, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood. 1997; 89(6): 2079–2088, indexed in Pubmed: 9058730.

24. Greenberg PL, Tuechler H, Schanz J, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood. 2012; 120(12): 2454–2465, doi: 10.1182/blood-2012-03-420489, indexed in Pubmed: 22740453.

25. Della Porta MG, Malcovati L, Strupp C, et al. Risk stratification based on both disease status and extra-hematologic comorbidities in patients with myelodysplastic syndrome. Haematologica. 2011; 96(3): 441–449, doi: 10.3324/haematol.2010.033506, indexed in Pubmed: 21134982.

26. Malcovati L, Porta MG, Pascutto C, et al. Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: a basis for clinical decision making. J Clin Oncol. 2005; 23(30): 7594–7603, doi: 10.1200/JCO.2005.01.7038, indexed in Pubmed: 16186598.

27. Bejar R, Stevenson K, Abdel-Wahab O, et al. Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med. 2011; 364(26): 2496–2506, doi: 10.1056/NEJMoa1013343, indexed in Pubmed: 21714648.