



## Guidelines

# Guidelines on myelodysplastic syndromes: Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular



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## Introduction

The guidelines project is a joint initiative of the Associação Médica Brasileira and the Conselho Federal de Medicina. It aims to bring together information in the scientific literature to standardize conduct in order to help decision-making during treatment. The data contained in the following articles were prepared by and are recommended by the Associação Brasileira

de Hematologia, Hemoterapia e Terapia Celular (ABHH). Even so, all possible medical approaches should be evaluated by the physician responsible for treatment depending on patient's characteristics and clinical status.

Description of the method used to gather evidence.

These guidelines are the result of a systematic review centered on the Evidence-Based Medicine movement, where clinical experience is integrated with the ability to critically

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analyze and rationally apply scientific information, thereby improving the quality of medical care.

Questions are structured using the Patient/Problem, Intervention, Comparison and Outcome (PICO) system, allowing the identification of keywords which were the basis of evidence-based search strategies in the major scientific databases [Medline (via PubMed), EMBASE, Central (Cochrane), Lilacs (via BVS)] and manual searches.

Degree of recommendation and level of evidence

A: Major experimental and observational studies

B: Minor experimental and observational studies

C: Case reports (non-controlled studies)

D: Opinion without critical evaluation based on consensus, physiological studies or animal models

These guidelines are subdivided into five articles:

Part 1: Laboratory workup to confirm diagnosis

Part 2: Classification systems

Part 3: Treatment of low-risk patients without the 5q deletion

Part 4: Treatment of low-risk patients with the 5q deletion

Part 5: Treatment of high-risk disease

## Part 1: Myelodysplastic Syndromes – Laboratory workup to confirm diagnosis

### Objective

This article presents the guidelines on the examinations that are needed to confirm the diagnosis of myelodysplastic syndromes.

#### PICO system

Using the PICO system, the P corresponds to patients with suspected myelodysplastic syndrome, I to the indication of examinations, and O to the outcomes (diagnosis).

Thus, 38 studies were found and selected to answer the clinical question ([Appendix I](#)).

What examinations are needed to confirm the diagnosis of myelodysplastic syndromes?

## Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of hematopoietic stem cell (HSC) clonal diseases resulting from a sequence of acquired genetic alterations with the formation of an anomalous and genetically unstable clone or clones, with potential to evolve to acute myeloid leukemia.

So far, there is no single reliable biological or genetic marker for diagnosis. Dysplastic alterations of peripheral blood (PB) and bone marrow (BM) are still fundamental for the diagnosis and classification of this group of diseases.

The detection of increased blasts facilitates diagnosis of the most advanced phases of the disease; however, in the early phases with minor morphological abnormalities, diagnosis is mainly based on the exclusion of other nonclonal cytopenias and diseases.

These clonal disorders may occur at any age, but they are more common in adults, with exponential increases after the 5th decade with a mean age at diagnosis of 70

years. Most patients acquire *de novo* clonal anomalies, with some acquiring somatic mutations after exposure to genotoxic agents, such as chemotherapy or radiotherapy for other neoplasms. Moreover, aging-associated inflammation (inflammaging) contributes to genetic instability and MDS predisposition.

The complete blood count with reticulocyte count and cytomorphological analysis are the vital first steps in the diagnosis of MDS. Isolated or combined, persistent for at least six months and unexplained cytopenias are common. Anemia, the most common cytopenia, is usually macrocytic, associated with a significant reduction in the reticulocyte count. All other causes of cytopenias/dysplasias should be excluded, as well as other clonal diseases and congenital abnormalities. Subsequently, a morphological analysis of BM cytological smears and an investigation of ring sideroblasts by Perls staining are performed. BM biopsy is complementary as it allows a better evaluation of the morphology and distribution of megakaryocytes, topography of other lineages, search for lymphoid aggregates and assessment of the degree of fibrosis by a standardized staining procedure. This is followed by classical and molecular cytogenetic analysis and immunophenotyping<sup>1</sup>(D).

## Extraction of the Results

### Morphological diagnosis

The main criteria in the morphological evaluation is the observation of dyspoiesis or cellular atypia found in at least 10% of the cells of the lineage under consideration<sup>2</sup>(D),<sup>3</sup>(B). The main finding in cytology is a loss of cell uniformity compared to normal cells. Loss of architectural organization of the hematopoietic sector compared to its normal structure is found by histology. No single morphological finding is sufficient for the diagnosis for MDS.

### Bone marrow cytology

Although cytopenia is seen in the PB, the BM is normocellular or hypercellular. Several features are observed in the erythroid lineage such as maturation delay, failures in hemoglobinization, asynchrony between nucleus and cytoplasmic maturation, megaloblastoid changes, anomalous mitoses, multinuclearity, nuclear chromatin bridges, nuclear budding and ring sideroblasts.

In the granulocytic lineage, one might observe: maturation delay translated by the accumulations of the phases of myeloblasts and myelocytes or even metamyelocytes with multiple isolated nuclei, asynchrony between nucleus and cytoplasmic maturation, giant metamyelocytes, degranulation (hypogranularity and/or heterogeneous distribution within the cytoplasm), the pseudo-Pelger Huet anomaly, nuclear anomalies (hyposegmentation), donut-shaped cells, and bizarre, atypical, aberrant immature forms. Auer rods or bodies may be found. The blasts count in the BM is important not only as a criterion for diagnosis and classification, but also as it has a strong impact on the prognosis. Type I, II and III myeloblasts may coexist in the same patient,

in the same sample and, in some cases, at different times. The megakaryocytic lineage is the most characteristic marker of dyspoiesis when present. Hypersegmentation, dumbbell-shaped nuclei, large or small mononuclear megakaryocytes and micro-megakaryocytes can be observed<sup>4</sup>(D).

### Bone marrow histology

Cellularity, established as the percentage of parenchyma and adipocytes, should be checked by histology allowing the classification of BM as hypocellular, normocellular, or hypercellular. Interpretation should consider the normal range for age. Abnormal distribution of cellularity may occur. Ectopia is the abnormal location of cells: megakaryocytes and erythroblasts in a paratrabecular location, clusters of myeloblasts and/or promyelocytes containing >5 cells in the intertrabecular region of marrow (ALIPs - Abnormal Localization of Immature Precursors). An evaluation of the reticulin network is important, as it has shown prognostic significance. Lymphoid aggregates are present in about 20% of cases<sup>5–7</sup>(D).

The hyperfibrotic subtype of MDS presents significant degrees of medullary fibrosis; European Consensus Grades II and III occur in 10% of cases and have a more aggressive clinical course often associated with excess blasts<sup>8</sup>(D),<sup>9,10</sup>(B). The diagnosis is always based on histology. As BM fibrosis is not exclusive to MDS but occurs in other myeloid diseases, an accurate clinical evaluation is recommended as well as a search for evidence of dyspoiesis in the PB; a biopsy imprint may help define this criterion as there is often insufficient material for cytological evaluations due to the fibrosis<sup>11</sup>(D). Focal fibrosis was observed in 17% of cases of a large cohort with only 5% having a diffuse and compact pattern<sup>12</sup>(B).

Hypoplastic MDS is characterized by cellularity ≤30% in over 60-year-old patients; it is more common after chemotherapy or radiotherapy. This subtype corresponds to 10–15% of the cases and usually demands special attention in the differential diagnosis with severe aplastic anemia, which may be decisive for correct conditioning before BM transplantation<sup>13</sup>(D),<sup>14–16</sup>(B). The distinction between hypoplastic MDS and aplastic anemia is challenging in terms of morphology.

### Recommendation

Although developments have undeniably changed the role and significance of morphology in hematological diagnosis given by the World Health Organization (WHO) 2008 and WHO 2016 classifications, morphology is still the starting point for accurate diagnosis.

## Conventional and Molecular Cytogenetics

Cytogenetic analysis plays an important role in determining clonality in patients with suspected MDS. Cytogenetic abnormalities are observed in 50–60% of patients, with the most common anomalies being del(5q), monosomy or deletion of

**Table 1 – Recurrent cytogenetic abnormalities and their frequency in myelodysplastic syndromes at diagnosis<sup>17</sup>(D)**

| Abnormality          | Frequency (%)  |
|----------------------|----------------|
| -5/5q-               | 10–40 (MDS-T)  |
| -7/7q-               | 10–50 (MDS -T) |
| +8                   | 10             |
| del(9q)              |                |
| i(17q)/t(17p)        | 3–5            |
| -13/13q-             | 3              |
| del(11q)             | 3              |
| del(12p) t(12p)      | 3              |
| del(20q)             | 5–8            |
| Idic(X)(q13)         | 1–2            |
| -Y                   | 5%             |
| t(11;16)(q23;p13,3)  | 3 (MDS -T)     |
| t(3;21)(q26.2;q22.1) | 2 (MDS -T)     |
| t(6;9)(p23;q34)      | <1             |
| t(2;11)(p21;q23)     | <1             |
| inv(3)(q21q26.2)     | <1             |
| t(1;3)(p35.3;q21.2)  | <1             |

MDS-T: Therapy-related myelodysplastic syndrome

\*Nullisomy Y (-Y), 8 trisomy and del(20q) are cytogenetic abnormalities that are not considered definitive evidence of MDS

7q, trisomy 8 and del(20q). The diagnosis of MDS can be made in the absence of morphological dysplasia when there are recurrent cytopenias and cytogenetic abnormalities as described in the WHO 2008 classification (Table 1)<sup>17</sup>(D). However, nullisomy Y (-Y), 8 trisomy, and del(20q) are cytogenetic abnormalities that, in the absence of dysplasia, are not considered definitive evidence of MDS as they can be seen in other myeloproliferative diseases<sup>4,17</sup>(D).

In the case of repeated failure of conventional cytogenetic investigations (G-band karyotyping) due to the absence or poor quality of metaphases, fluorescence in situ hybridization (FISH) may complement the cytogenetic analysis.

BM samples from 48 MDS patients (43 low risk) were evaluated by karyotyping and FISH using probes for chromosomes 5, 7, 8, 11, 13 and 20. Eighteen of the 48 samples (37.5%) had an abnormal clone identified by metaphase chromosomal analysis, while 17 of 48 (35.5%) had an abnormal clone detected by FISH analysis. Normal karyotypes were detected in 30 of the 48 patients (62.5%) with 29 of these 30 having a normal FISH result. The sensitivities of the two methodologies (FISH and karyotyping) were very similar and so FISH may be a good tool to study BM aspirate when cytogenetic analysis is not available<sup>18,19</sup>(B).

In another study, 433 BM samples from patients with suspected MDS or AML were submitted to karyotyping and FISH for -5/5q-, -7/7q-, +8 and 20q-. Abnormal karyotypes were identified in 114 cases (26.3%) and abnormal FISH results of at least one locus were found in 94 cases (21.7%). Abnormal results by FISH or karyotyping were detected in 136/433 samples (31.4%). Of the 136 cases with any abnormal finding, 22 (16.2%) were identified only by FISH. The two methods had concordance in 96.5%, 96.5%, 93.9% and 93% of the cases of abnormalities of chromosomes 5, 7, 8 and 20, respectively<sup>20</sup>(B).

## Molecular genetics

Array-based karyotyping is a novel technique that allows the detection of chromosomal abnormalities not observed by conventional cytogenetics, chiefly with the use of single nucleotide polymorphism arrays (SNP-arrays)<sup>21</sup>(D),<sup>22</sup>(B). Somatic mutations are observed in 80–90% of MDS patients with the most commonly affected genes being *SF3B1*, *TET2*, *SRSF2*, *ASXL1*, *DNMT3A*, *RUNX1*, *U2AF1*, *TP53* and *EZH2*. However, these mutations, in addition to being observed in other neoplasms, in particular myeloid neoplasms, may also occur in apparently healthy elderly patients as the so-called ‘clonal hematopoiesis of indeterminate potential’ (CHIP)<sup>23</sup>(D). Although some patients may progress to MDS, the natural course of these cases has not yet been elucidated and the presence of these somatic mutations associated with MDS was not included as a diagnostic criterion in the WHO 2016 classification<sup>4</sup>(D). As with cytogenetic abnormalities, the type and number of mutations are associated with the evolution/progression of MDS. Mutations of *TP53* are associated with the most aggressive type of disease and, in these cases, evaluation is indicated, particularly in respect to MDS with isolated del(5q), which determines a less favorable prognosis and seems to predict an unfavorable response to lenalidomide<sup>24</sup>(B).

The *SF3B1* mutation was described in 30% of MDS cases, and in 80% of MDS with ring sideroblasts, that is, this mutation is predictive of ring sideroblasts<sup>25</sup>(B). According to the WHO 2016 classification, cases of suspected MDS who have between 5 and 15% of ring sideroblasts should be investigated for this mutation with the diagnosis being conclusive when it is present.

Generally, patients with MDS with ring sideroblasts and *SF3B1* mutations (most frequently in exon 15 - K700E) are elderly with low-risk international prognostic score (IPSS) who present better overall survival, greater event-free survival and favorable karyotypes<sup>26</sup>(B). Patients with MDS with ring sideroblasts and no *SF3B1* mutations in general present *TP53* mutations and worse outcomes.

## Multiparametric flow cytometry in the diagnosis of myelodysplastic syndromes and search for prognostic factors

Immunophenotyping by flow cytometry (FCM) allows an analysis of the antigen expression related to lineage and degree of maturation in normal and abnormal hematopoiesis<sup>27</sup>(B). The expression of this antigen is tightly controlled by genetic and epigenetic mechanisms, leading to a predictable normal pattern at different stages of maturation<sup>28</sup>(B). In MDS, there is abnormal granulocytic, monocytic, and erythroid differentiation resulting in deviations from the normal pattern. There may be an increase or decrease in antigen expression, asynchronous expression, or aberrant cross-lineage antigen expression. This can be detected by FCM. Thus, this technique is an important ancillary tool in the diagnosis of MDS cases where few morphological abnormalities are found in BM cytology and histology and the karyotype is normal<sup>28–31,33</sup>(B),<sup>32,34</sup>(D).

## Recommendations

Conventional cytogenetic analysis (G-band karyotyping) is an integral part of the evaluation of patients with suspected MDS. The results may not be informative when an insufficient number of cell metaphases (<20 metaphases) is obtained due to hypocellularity or in the absence of cell growth.

FISH studies, in particular chromosomes 5, 7, 8 and 20, are useful in patients for whom several cultures of bone marrow were performed without obtaining metaphases. This study may also help in the elucidation of complex chromosomal abnormalities. FISH analysis should not replace conventional karyotyping as an analysis of the whole set of chromosomes is essential.

Array-based karyotyping is complementary to metaphasic karyotyping and can be added to diagnostic tests however it is expensive and not widely available in Brazil. In addition, the detection of somatic mutations, even though observed in 80–90% of MDS cases, does not yet have a clear role in the diagnostic evaluation of unexplained cytopenias. Mutation screening of *TP53* is indicated particularly in MDS patients with del(5q) and screening for *SF3B1* mutations in patients with between 5–14% of ring sideroblasts in the BM.

In the last 15 years, numerous papers have been published about this subject, and international study groups, such as the European LeukemiaNet, have standardized this analysis for 4- and 8-color panels<sup>32</sup>(D),<sup>33</sup>(B). In contrast to morphology, that is susceptible to subjective evaluation, FCM analysis is an objective method if well standardized. Furthermore, in cytological analysis it is recommended to count 500 cells, while in FCM at least 100,000 cells (and >250 CD34<sup>+</sup> cells) should be analyzed for the diagnosis of MDS. Immunophenotyping of BM cells is able to detect numerous maturation abnormalities in the myelomonocytic and erythroblastic lineages as well as analysis of myeloid and lymphoid progenitors<sup>34</sup>(B).

Increases of myeloid CD34<sup>+</sup> cells often presenting aberrant and cross-lineage antigen expressions may be found, especially in refractory anemia with excess of blasts (RAEB)<sup>29–31,34–38</sup>(B). Moreover, B-cell precursor counts (hematogones type I) are lower compared to the normal number for the age of the patient<sup>33,39</sup>(B).

No fixed antibody panels or combinations have been recommended<sup>32</sup>(D),<sup>34</sup>(B). The analysis performed must always be compared to the findings of age-matched normal BM (BM donors, orthopedic surgery patients, etc.)<sup>32</sup>(D),<sup>29,31,33–35,39</sup>(B). FCM abnormalities found should be interpreted in the context of the clinical findings and other laboratory results, such as BM cytology and cytogenetics. There is no single pathognomonic abnormality for MDS. International recommendations establish that all abnormalities should be reported and the diagnosis of MDS is suspected if at least two aberrations are found.

Several efforts have been made to establish diagnostic scores but there is much controversy in the literature concerning this subject and none of the described scores is universally accepted<sup>21</sup>(D),<sup>33</sup>(B). The most commonly used in

the clinical practice is the Flow Cytometry-based Scoring System (FCSS)<sup>40</sup>(B) based on several parameters of maturation abnormalities of the myelomonocytic series and increases and phenotypic aberrancies of myeloid progenitors. A simpler score (Ogata score) is based on four parameters: CD34<sup>+</sup> myeloblast-related and B-progenitor-related cluster sizes, CD45 expression of myeloblasts and granulocyte side scatter using the values obtained for these parameters in the lymphocyte gate as an internal control. This score has a sensitivity of 70% and a specificity of 93% for the diagnosis of MDS<sup>41</sup>(B).

In earlier studies there was a concern to compare the findings of FCM with those of BM cytology and cytogenetics<sup>28,30,36</sup>(B). However, in more recent papers, as FCM has been well standardized, the emphasis is placed on a comparison with normal age-matched BM and how much the variables obtained in MDS differ from normal findings. Thus, it is important that each laboratory working in this field has its own local normal control values.

Nowadays, immunophenotyping in MDS has been well standardized, and based on the knowledge of the normal antigen expression of the several hematopoietic precursors in BM. Usually one or two standard deviations from the normal mean fluorescence intensity is used to say that there is an abnormal expression of an antigen. Also, it is more important to have local normal values of CD34<sup>+</sup> cells rather than to use fixed normal values described in the literature, as these values may change with the fluorochromes conjugated to the monoclonal antibodies, the gating strategy used, quality controls of each laboratory as well as patients' age<sup>31</sup>(B),<sup>32</sup>(D). This technique demands good training of the operator but it is easily feasible in a routine laboratory and is useful to discriminate between reactive (non-clonal) peripheral cytopenias and low grade MDS with few BM cell atypias<sup>29,33,40,41</sup>(B).

FCM analysis of BM cells is also able to provide prognostic parameters, associated with survival, response to treatment and BM transplantation<sup>32,36,40</sup>(B),<sup>42</sup>(D). In a cohort of 101 Brazilian patients, the number of CD34<sup>+</sup> myeloblast-related cells was an independent prognostic parameter compared to the WHO Classification-Based Prognostic Scoring System (WPSS) and Revised IPSS (IPSS-R), and had higher prognostic value than IPSS.

FCM is considered an ancillary technique in the diagnosis of MDS and should always be reported and interpreted together with the PB values, BM cytology and cytogenetics which remain the gold standard for diagnosis<sup>29</sup>(B). However, when the karyotype is normal, and cytology is inconclusive, FCM is a valuable technique to confirm the diagnosis<sup>4</sup>(D).

### Recommendations

Immunophenotyping of BM hematopoietic precursors should be performed in patients with peripheral cytopenias stable for at least six months where BM morphology and cytogenetics do not give a clear-cut diagnosis. It is also useful to evaluate prognosis of the patient and to monitor response to treatment.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Appendix I.

### 1. Clinical question

What examinations are needed to confirm the diagnosis of myelodysplastic syndromes?

### 2. Structured question (PICO)

Patient Patients with MDS

Intervention Bone marrow aspiration with iron staining (Perls)

Bone marrow biopsy (hematoxylin-eosin and reticulin)

Bone marrow karyotype

Fluorescence in situ hybridization (FISH) panel (monosomy/deletion of chromosomes 5 and 7 and trisomy 8)

Bone marrow immunophenotyping by flow cytometry to investigate quantitative alterations and alterations of antigen expression in hematopoietic precursors of the bone marrow

Comparison None

Outcome Diagnosis, confirmation

### 3. Initial eligibility criteria for studies

- Components of PICO
- No time limit
- No limit of languages
- Full text availability

### 4. Search strategies

- #1: (Myelodysplastic Syndrome OR Myelodysplastic Syndromes OR Dysmyelopoietic Syndromes OR Dysmyelopoietic Syndrome OR Hematopoietic Myelodysplasia OR Hematopoietic Myelodysplasias)= 22.751
- #2: (Myeloid Neoplasms OR Pancytopenia OR Anemia, Refractory OR Lymphoid Neoplasms OR Primary Myelofibrosis OR Bone Marrow = 362.675

### 5. Selection of articles

Initially selected by the title, sequentially by the abstract, and finally by the full text, the latter being subjected to critical evaluation and extraction of outcomes related to the outcomes.

### 6. Critical evaluation and strength of evidence

The strength of the evidence of the studies was defined taking into account the study design and the corresponding risks of bias, the results of the analysis (magnitude and precision), relevance and applicability (Oxford/GRADE).<sup>43,44</sup>

## references

1. Niero-Melo L, Resende LS, Gaiolla RD, Oliveira CT, Domingues MA, Moraes Neto FA. Guidelines for morphological diagnosis of myelodysplastic syndromes. *Rev Bras Hematol Hemater.* 2006;28(3):167–74.

2. Bennett JM, Komrokji RS. The Myelodysplastic syndromes: diagnosis, molecular biology and risk assessment. *Hematology*. 2005;10 suppl 1:258–69.
3. Ramos F, Fernández-Ferrero S, Suárez D, Barbón M, Rodríguez JA, Gil S, et al. Myelodysplastic syndrome: a search for minimal diagnostic criteria. *Leuk Res*. 1999;23(3):283–90.
4. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391–405.
5. Bartl R, Frisch B, Baumgart R. Morphologic classification of the myelodysplastic syndromes (MDS): combined utilization of the bone marrow aspirates and trephine biopsies. *Leuk Res*. 1992;16(1):15–33.
6. Yuhua S, Shuling Q, Laiquan L, Chongli Y. Studies on micromegakaryocytes in myelodysplastic syndromes (MDS). *Proc CAMS and PUMC*. 1988;3(1):33–9.
7. Tuzuner N, Cox C, Rowe JM, Bennett JM. Bone marrow cellularity in myeloid stem-cell disorders: impact of age correction. *Leuk Res*. 1994;18(8):559–64.
8. Thiele J, Kvasnicka HM, Facchetti F, Franco V, van der Walt J, Orazi A. European consensus on grading bone marrow fibrosis and assessment of cellularity. *Hematologia*. 2005;90(8):1128–32.
9. Lambertenghi-Delilieri G, Orazi A, Luksch R, Annaloro C, Soligo D. Myelodysplastic syndrome with increased marrow fibrosis: a distinct clinico-pathological entity. *Br J Haematol*. 1991;78(2):161–6.
10. Della Porta MG, Malcovati L, Boveri E, Travaglini E, Pietra D, Pascutto C, et al. Clinical relevance of bone marrow fibrosis and CD34-positive cell clusters in primary myelodysplastic syndromes. *J Clin Oncol*. 2009;27(5):754–62.
11. Rosati S, Anastasi J, Vardiman J. Recurring diagnostic problems in the pathology of the myelodysplastic syndromes. *Semin Hematol*. 1996;33(2):111–26.
12. Maschek H, Georgii A, Kaloutsi V, Werner M, Bandekar K, Kressel MG, et al. Myelofibrosis in primary myelodysplastic syndromes: a retrospective study of 352 patients. *Eur J Haematol*. 1992;48(4):208–14.
13. Greenberg PL, Young NS, Gattermann N. Myelodysplastic syndromes. *Hematol Am Soc Hematol Educ Program*. 2002;136–61.
14. Orazi A, Albitar M, Heerema NA, Haskins S, Neiman RS. Hypoplastic myelodysplastic syndromes can be distinguished from acquired aplastic anemia by CD34 and PCNA immunostaining of bone marrow biopsy specimens. *Am J Clin Pathol*. 1997;107(3):268–74.
15. Baumann I, Fu M, Behrendt S, Campr V, Csomor J, Furlan I, et al. Morphological differentiation of severe aplastic anaemia from hypocellular refractory cytopenia of childhood: reproducibility of histopathological diagnostic criteria. *Histopathology*. 2012;61(1):10–7.
16. Fohlmeister I, Fischer R, Mödder B, Rister M, Schaefer HE. Aplastic anaemia and the hypocellular myelodysplastic syndrome: histomorphological, diagnostic, and prognostic features. *J Clin Pathol*. 1985;38(11):1218–24.
17. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: IARC; 2008. p. 439.
18. Cherry AM, Brockman SR, Paternoster SF, Hicks GA, Neuberger D, Higgins RR, 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):1085–90.
19. Pinheiro RF, Chauffaille ML. Comparison of I-FISH and G-banding for the detection of chromosomal abnormalities during the evolution of myelodysplastic syndrome. *Braz J Med Biol Res*. 2009;42(11):1110–2.
20. Coleman JF, Theil KS, Tubbs RR, Cook JR. Diagnostic yield of bone marrow and peripheral blood FISH panel testing in clinically suspected myelodysplastic syndromes and/or acute myeloid leukemia: a prospective analysis of 433 cases. *Am J Clin Pathol*. 2011;135(6):915–20.
21. Malcovati L, Hellström-Lindberg E, Bowen D, Adès L, Cermak J, Del Cañizo C, et al. Diagnosis and treatment of primary myelodysplastic syndromes in adults recommendations from the European LeukemiaNet. *Blood*. 2013;122(17):2943–64.
22. Tiu RV, Gondek LP, O'Keefe CL, Elson P, Huh J, Mohamedali A, et al. Prognostic impact of SNP array karyotyping in myelodysplastic syndromes and related myeloid malignancies. *Blood*. 2011;117(17):4552–60.
23. Steensma DP, Bejar R, Jaiswal S, Lindsley RC, Sekeres MA, Hasserjian RP, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood*. 2015;126(1):9–16.
24. Jädersten M, Saft L, Smith A, Kulasekararaj A, Pomplun S, Göhring G, et al. TP53 mutations in low-risk myelodysplastic syndromes with del(5q) predict disease progression. *J Clin Oncol*. 2011;29(15):1971–9.
25. Malcovati L, Papaemmanuil E, Bowen DT, Boulwood J, Della Porta MG, Pascutto C, et al. Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. *Blood*. 2011;118(24):6239–46.
26. Lavallée VP, Faucher D, Lamontagne B, Hébert J, Busque L. SF3B1 Mutations Are Associated with Favorable Cytogenetics in Patients with myelodysplastic Syndromes with Ring Sideroblasts. *Blood*. 2012;120. Abstract 3823.
27. van Lochem EG, van der Velden VH, Wind HK, te Marvelde JG, Westerdaal NA, van Dongen JJM. Immunophenotypic differentiation patterns of normal hematopoiesis in human bone marrow: reference patterns for age-related changes and disease-induced shifts. *Cytometry B Clin Cytom*. 2004;60(1):1–13.
28. Stetler-Stevenson M, Arthur DC, Jabbour N, Xie XY, Mollidrem J, Barrett AJ, et al. Diagnostic utility of flow cytometric immunophenotyping in myelodysplastic syndrome. *Blood*. 2001;98(4):979–87.
29. Truong F, Smith BR, Stachurski D, Cerny J, Medeiros LJ, Woda BA, et al. The utility of flow cytometric immunophenotyping in cytopenic patients with a non-diagnostic bone marrow: a prospective study. *Leuk Res*. 2009;33(8):1039–46.
30. Stachurski D, Smith BR, Pozdnyakova O, Andersen M, Xiao Z, Raza A, et al. Flow cytometric analysis of myelomonocytic cells by a pattern recognition approach is sensitive and specific in diagnosing myelodysplastic syndrome and related marrow diseases: emphasis on a global evaluation and recognition of diagnostic pitfalls. *Leuk Res*. 2008;32(2):215–24.
31. Lorand-Metze I, Ribeiro E, Lima CSP, Batista LS, Metze K. Detection of hematopoietic maturation abnormalities by flow cytometry in myelodysplastic syndromes and its utility for the differential diagnosis with non-clonal disorders. *Leuk Res*. 2007;31(2):147–55.
32. Van de Loosdrecht AA, Alhan C, Bene MC, Della Porta MG, Drager AM, Feuillard J, et al. Standardization of flow cytometry in myelodysplastic syndromes: report from the first European LeukemiaNet working conference on flow cytometry in myelodysplastic syndromes. *Haematologica*. 94(8):1124–34.
33. Ogata K, Della Porta MG, Malcovati L, Picone C, Yokose N, Matsuda A, et al. Diagnostic utility of flow cytometry in low-grade myelodysplastic syndromes: a prospective validation study. *Hematologia*. 2009;94(8):1066–74.
34. Westers TM, Ireland R, Kern W, Alhan C, Balleisen JS, Bettelheim P, et al., Standardization of flow cytometry in myelodysplastic syndromes: a report from an international

- consortium and the European LeukemiaNet Working Group. *Leukemia*. 2012;26(7):1730–41.
35. Malcovati L, Della Porta MG, Lunghi M, Pascutto C, Vanelli L, Travaglino E, et al. Flow cytometry evaluation of erythroid and myeloid dysplasia in patients with myelodysplastic syndrome. *Leukemia*. 2005;19(5):776–83.
  36. Kern W, Haferlach C, Schnittger S, Haferlach T. Clinical utility of multiparameter flow cytometry in the diagnosis of 1013 patients with suspected myelodysplastic syndrome: correlation to cytomorphology, cytogenetics, and clinical data. *Cancer*. 2010;116(19):4549–63.
  37. De Smet D, Trullemans F, Jochmans K, Renmans W, Smet L, Heylen O, et al. Diagnostic potential of CD34+ cell antigen expression in myelodysplastic syndromes. *Am J Clin Pathol*. 2012;138(5):732–43.
  38. Goardon N, Nikolousis E, Sternberg A, Chu WK, Craddock C, Richardson P, et al. Reduced CD38 expression on CD34+ cells as a diagnostic test in myelodysplastic syndromes. *Hematologia*. 2009;94(8):1160–3.
  39. Reis-Alves SC, Traina F, Metze K, Lorand-Metze I. Improving the differential diagnosis between myelodysplastic syndromes and reactive peripheral cytopenias by multiparametric flow cytometry: the role of B-cell precursors. *Diagn Pathol*. 2015;10:44.
  40. Chu SC, Wang TF, Li CC, Kao RH, Li DK, Su YC, et al. Flow cytometric scoring system as a diagnostic and prognostic tool in myelodysplastic syndromes. *Leuk Res*. 2011;35(7):868–73.
  41. Della Porta MG, Picone C, Pascutto C, Malcovati L, Tamura H, Handa H, et al. Multicenter validation of a reproducible flow cytometric score for the diagnosis of low-grade myelodysplastic syndromes: results of a European LeukemiaNET study. *Hematologia*. 2012;97(8):1209–17.
  42. Reis-Alves SC, Traina F, Harada G, Campos PM, Saad ST, Metze K, et al. Immunophenotyping in myelodysplastic syndromes can add prognostic information to well-established and new clinical scores. *PLOS One*. 2013;8:e81048.
  43. Goldet G, Howick J. Understanding GRADE: an introduction. *J Evidence Based Med*. 2013;6:50–4.
  44. Levels of Evidence and Grades of Recommendations - Oxford Centre for Evidence Based Medicine. Available from URL: [http://cebm.jr2.ox.ac.uk/docs/old\\_levels.htm](http://cebm.jr2.ox.ac.uk/docs/old_levels.htm)