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Progress in diagnostic staging of myelodysplastic syndrome

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Abstract: *Myelodysplastic syndromes (MDS) are a group of acquired heterogeneous clonal disorders that originate from hematopoietic stem cells. It is characterized by a reduction in one or more blood lines in the myeloid system with abnormal development, pathological hematopoiesis and a high risk of transformation to acute myeloid leukemia (AML). In 2016, the World Health Organization (WHO) revised the diagnostic staging criteria for MDS, but with the development of next-generation sequencing (NGS) technology, the diagnostic staging of MDS has opened a new era of accurate diagnosis and individualized treatment. In this paper, we review the diagnostic typing of MDS.*

Keywords: *Myelodysplastic syndrome; diagnostic typing; next-generation sequencing technology; precise diagnosis*

1. Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of myeloid clonal hematopoietic stem cell disorders characterized by a reduction of one or more blood lines in the myeloid system with abnormal development, pathological hematopoiesis, and a high risk of transformation to acute myeloid leukemia (AML). With the development of next-generation sequencing (NGS) technology, mutations in genes involved in nuclear DND methylation regulation, histone modifications, RNA spliceosomes, transcription factors and signal transduction pathways have been found to be involved in the pathogenesis of MDS. The most frequently affected genes are SF3B1, TET2, ASXL1, SRSF2, RUNX1, TP53, U2AF1 and EZH2 [1]. The study of these gene mutations is of great significance to promote the accurate diagnosis and individualized treatment of MDS. In this paper, we review the research progress of MDS diagnostic typing.

2. Diagnosis

2.1. Minimum diagnostic criteria

The current minimum diagnostic criteria for MDS use the 2016 WHO criteria. Required conditions: (1) Persistent mono- or multilineage hematocrit for at least 4 months (MDS is diagnosed in the presence of primipocytosis or MDS-related cytogenetic abnormalities). (2) Exclusion of other blood disorders and other diseases that may cause hematocrit and developmental abnormalities. Among the criteria for hematocrit reduction are: absolute neutrophil value < 1.8×109/L, hemoglobin < 100 g/L, and platelet count < 100×109/L. ajor criteria: (1) The proportion of abnormal cells of any one or more of the red, granulocyte, and megakaryocyte lineages in the bone marrow smear was ≥ 10%. (2) ≥ 15% cyclic iron granulocytes (in nucleated erythrocytes) or ≥ 5% with SF3B1 gene mutation. (3) Bone marrow smear primitive cells of 5%-19% (or peripheral blood smear primitive cell count of 2%-19%). (4) Typical chromosomal abnormalities detected by conventional karyotype analysis or fluorescence in situ hybridization (FISH). Ancillary criteria: (1) Bone marrow pathology biopsy or immunohistochemistry results support the diagnosis of MDS. (2) Flow Cytometry (FCM) detects multiple MDS-associated immunophenotypic abnormalities, supporting the presence of monoclonal red and/or myeloid cell populations. (3) Detection of MDS-associated genetic mutations by molecular biology methods (gene sequencing techniques), supporting clonal hematopoiesis. MDS can be diagnosed by meeting two of the

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required conditions and at least one of the main criteria. Suspected MDS can also be diagnosed if the two necessary conditions are met but not the main criteria, with typical clinical manifestations of MDS and at least two ancillary criteria [2].

2.2. Diagnosis method

2.2.1. Cytomorphological testing

Morphological abnormalities in peripheral blood and bone marrow smears of MDS patients are manifested by an increased proportion of primitive cells and abnormal development [3]. Domestic scholars have emphasized that cytomorphological testing for MDS includes not only routine cytomorphological analysis under light microscopy but also special cellular and histomorphological staining such as cytochemical staining, immunohistochemical staining, Prussian blue staining and silverophilic staining [4]. Furthermore, studies have shown that bone marrow pathology biopsy is superior to bone marrow smear in assessing the degree of bone marrow proliferation, bone marrow fibrosis, and bone marrow hematopoietic tissue structure [5, 6]. Therefore, the inclusion of cytochemical staining of bone marrow smears and peripheral blood smears, immunohistochemical staining analysis of CD61 monoclonal antibody in bone marrow histiocytes and bone marrow pathological biopsy as essential tests for all patients with suspected MDS is of great significance for the accurate diagnosis of MDS.

2.2.2. Cytogenetic testing

One of the main manifestations of clonal hematopoiesis in MDS is the non-random karyotypic abnormalities that can be detected by routine karyotype analysis [3]. Common non-random karyotypic abnormalities currently available include +8, -7/del (7q), del (20q), - 5/del (5q) and -Y, but lack specificity for the diagnosis of MDS. Analysis of at least 20 conventional karyotypic dominant bands (G- and R-bands) of myeloid cells for mid-life divisions and karyotype reporting according to the 2013 International System for Human Cytogenetic Nomenclature (ISCN) guidelines is often required [7]. However, this can be complemented by FISH testing with a combination of probes in patients with suspected MDS who have a low degree of myeloproliferation, dry bone marrow, no intermediate schizotypes, or less than 20 intermediate schizotypes. In addition, gene microarray techniques such as single nucleotide polymorphism-microarray comparative genomic hybridization (SNP-array) are often included as complementary tests to conventional karyotyping because of their advantage of detecting abnormal DNA copy number and uniparental diploidy in most MDS patients and further improving the detection rate of cytogenetic abnormalities in MDS patients [4].

2.2.3. Immunological testing

Currently, there are no MDS-specific antigenic markers or combinations of markers. The diagnosis of MDS cannot be established solely on the basis of FCM results in patients who lack clear diagnostic significance in terms of cytomorphological or cytogenetic manifestations. However, FCM is of high value in the prognostic stratification of MDS and in the differential diagnosis of low-risk MDS and non-clonal hemocytopenia. In patients without typical morphologic and cytogenetic evidence and without a diagnosis of MDS, FCM test results can be used as one of the auxiliary diagnostic criteria [7]. In China, some scholars have studied the flow cytometric scoring system (FCSS) that applies flow cytometry results to the diagnosis of MDS. They detected different immunophenotypic abnormalities of bone marrow cells and scored them. The score was increased by 1, 2, 3 and 4 points for abnormal myeloid primitive cells < 5% (or lympho/myeloid ratio > 1.0), 5% to 10%, 11% to 20% and 21% to 30%, respectively. The results showed that the specificity of FCSS for the diagnosis of MDS was 90.25% and the sensitivity was 85.40% when the FCSS score was ≥2 [8]. Therefore, FCSS is more practical, and further study of MDS-specific antigenic marker profile is of great clinical significance for the accurate diagnosis of MDS.

2.2.4. Molecular genetic testing

Genetic mutations are another major manifestation of clonal hematopoiesis in MDS [3]. In recent years, with the development of next-generation sequencing technology, some new knowledge and understanding of the pathogenesis, diagnosis, treatment and prognosis of MDS have been obtained through the study of MDS genomics, extensive sequencing and data summarization [9]. The application of NGS for gene mutation detection in patients with suspected MDS can detect at least one gene mutation, which is potentially valuable for the clinical diagnosis of MDS. For example, the SF3B1 gene mutation has important diagnostic and differential diagnostic value for the MDS with annular iron granulocyte-erythrocyte (MDS-RS) subtype. The WHO's 2016 MDS diagnostic criteria suggest that the SF3B1 gene

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should be a mandatory gene for all patients with suspected MDS [2]. In addition, the latest international and domestic MDS diagnostic guidelines have now included NGS technology as a mandatory test for MDS diagnosis, which is conducive to the promotion of accurate diagnosis of MDS [7, 10-12].

3. Typing criteria

3.1. FAB typing criteria

In 1982, the French-American-Britain (FAB) Collaborative Group first proposed diagnostic typing criteria for MDS based on cytomorphology, which classified MDS into five types. (1) Refractory anemia (RA): peripheral blood primitive cells < 1%, bone marrow primitive cells < 5%. (2) Refractory anemia with ring sideroblasts (RARS): peripheral blood primitive cells < 1%, bone marrow primitive cells < 5%, ring sideroblasts > 15% of nucleated red blood cells. (3) Refractory anemia with an excess of blast (RA with RAEB): < 5% peripheral blood primitive cells, 5%-20% bone marrow primitive cells. (4) Refractory anemia with an excess of blast in transformation (RAEB-T): ≥ 5% peripheral blood blasts, 20%-30% bone marrow blasts, or Auer vesicles in young granulocytes. (5) chronic myelomonocytic leukemia (CMML): < 5% peripheral blood primitive cells, > 1×109/L absolute monocytes, and 5%-20% bone marrow primitive cells [13]. This typing criterion has been replaced by the later WHO diagnostic typing criteria because of its limitations for current clinical practice.

3.2. WHO typing criteria

The WHO diagnostic staging criteria for MDS based on cytomorphology, cell biology and genetics replaced the more limited FAB diagnostic staging criteria in 2001, and were revised twice in 2008 and 2016. By continuously updating, revising and improving the diagnostic staging criteria for MDS, the current MDS diagnostic staging model integrates morphology, immunology, cytogenetics, and molecular biology (MICM). It can better guide the precise diagnosis and individualized treatment of clinical MDS [4].

In 2016, the main changes of the latest WHO revised MDS diagnostic staging criteria include: (1) the names of "refractory anemia" and "refractory hematocrit" were removed and replaced by MDS. (2) Emphasis was placed on the fact that the percentage of primary cells in the bone marrow and peripheral blood smear counts cannot be replaced by the percentage of CD34+ cells in the FCM test. (3) Revised the diagnostic criteria for MDS with ring sideroblasts (MDS-RS): the presence of the SF3B1 mutation and the presence of ≥ 5% ring sideroblasts alone is diagnostic. (4) Addition of relevant karyotype analysis. (5) The cytogenetic criteria for MDS with simple del(5q) were revised: it can be accompanied by 1 other cytogenetic abnormality [except -7 or del(7q)]. (6) The rule of calculating the proportion of primitive cells in non-erythroid cells was removed and the classification of AML or MDS was based only on the calculation of the proportion of primitive cells occupying nucleated cells [3].

3.2.1. Typing nomenclature

WHO constantly updates, revises and refines the diagnostic staging criteria for MDS based on the recommendations of the Clinical Advisory Committee. The WHO diagnostic staging criteria mainly emphasize the coefficient of developmental abnormalities and the number of primitive cells rather than hematocrit. However, in the 2001 and 2008 WHO diagnostic staging criteria, "hematocrit" and "anemia" are present in the names of almost all subtypes. At the same time, in refractory hemocytopenia with unilineage developmental abnormalities, the type of developmental abnormalities and hemocytopenia are sometimes inconsistent. Therefore, in 2016, the WHO dropped the names "refractory anemia" and "refractory hematocrit" and replaced them with MDS. Refractory anemia (RA), refractory neutropenia (RN), and refractory thrombocytopenia (RT) subtypes have been unified under the name MDS with single lineage dysplasia (MDS). MDS with single lineage dysplasia (MDS-SLD). Refractory anemia with ring sideroblasts (RARS) was renamed MDS with single lineage dysplasia (MDS-RS with single lineage Dysplasia, MDS-RS-SLD). Refractory cytopenia with multilineage dysplasia (RCMD) was reclassified as MDS-RS with multilineage dysplasia (MDS-RS MDS with multilineage dysplasia (MDS-RS) and MDS with multilineage dysplasia (MDS-MLD). Refractory anemia with primocytosis types 1 and 2 (RAEB-1 and RAEB-2) were renamed MDS with primocytosis types 1 and 2 (MDS-EB1 and MDS-EB2) [3].

3.2.2. Morphology

The criteria for the evaluation of the primitive cell count are still based on morphology, the FCM

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assay results lack specificity, and the CD34+ cell ratio results have the potential for misclassification. Therefore, the 2016 WHO revision emphasizes that the primitive cell ratio of bone marrow and peripheral blood smear sorting counts cannot be replaced by the CD34+ cell ratio of the FCM assay, but only as an auxiliary criterion. Although a morphologic threshold of 10% is still used for developmental abnormalities, more specific morphologic criteria for developmental abnormalities exist for different lineages of bone marrow cells due to the different sensitivities of each lineage. The International Prognostic Scoring System Revised (IPSS-R) recommends a 2% primitive cell ratio to define different risk groups. However, in clinical practice, it is difficult to distinguish between 0-2% and 2%-5% primitive cell proportions. Therefore, in the 2016 WHO revision, the 2% threshold was not adopted, but only emphasized that the specific primitive cell percentage should be indicated in the patient's bone marrow report, not just indicating a bone marrow primitive cell < 5%. It has also been suggested to increase the threshold of primitive cells by modifying < 20% to < 30%. There is no consensus on whether to diagnose them as MDS or AML because for the fraction of patients with 20% to 30% primitive cells, their disease progresses slowly and is effectively treated with MDS-like therapy. However, the 2016 WHO revision was not adopted, and the original criteria remained unchanged. In MDS-U with mono- or multilineage developmental abnormalities, the threshold value of 1% of primitive cells in peripheral blood was not considered in previous clinical work. Therefore, the 2016 WHO revision emphasizes that peripheral blood primitive cells = 1% must be documented on two separate occasions in order to classify MDS with unilineage or multilineage developmental abnormalities as MDS-U (MDS, unclassifiable). MDS-U also includes MDS with unilineage abnormal hematopoiesis with allogeneic cytopenia and MDS with diagnostically significant karyotype abnormalities.

In addition, the 2016 WHO revision abolished the rule of calculating the proportion of primitive cells to non-erythroid cells and classified AML or MDS only according to the proportion of primitive cells to all nucleated cells (ANC). This change is mainly reflected in the diagnostic staging of acute red leukemia. The 2008 WHO revision classifies erythroleukemia (erythroid/myeloid) as AML, defined as ≥ 50% erythroid in the bone marrow and ≥ 20% non-erythroid primitive cells in the bone marrow. However, patients with < 20% of all nucleated cells in the bone marrow were classified as having MDS based on the proportion of primitive cells to all nucleated cells. In the 2016 WHO revision, the original calculation rule was abolished and the name was changed to MDS with primitive cellular increase (MDS-EB) [3].

3.2.3. Immunophenotyping

Studies have shown that abnormal cellular immunophenotypes have good sensitivity and specificity for the diagnosis of MDS. The MDS flow cytometry immunophenotyping scoring system has made great progress in recent years. FCM immunophenotyping focuses on five aspects: myeloid progenitor cells, neutrophils, monocytes, erythrocytes and B progenitor cells to determine whether there are abnormal developmental changes. Currently, two systems are proposed internationally.

(1) Ogata is mainly used to determine granulocyte monomyeloid changes. In 2009, Ogata proposed a four-parameter scoring system, which are CD34+ myeloid primitive cell ratio, CD34+ B progenitor cell ratio, lymphocyte to myeloid primitive cell CD45 ratio, and granulocyte to lymphocyte SSC ratio. This score has a sensitivity < 70% for the diagnosis of MDS, but a specificity of 90%. It is very helpful in the diagnosis of MDS in patients with uncertain morphology and cytogenetics [14]. In 2018 Ogata updated the 5-point scale by increasing the ratio of CD34+ Myeloid primitive cells CD33. Its sensitivity was not significantly improved and was only about 50% [15]. Overall, this scoring method is simpler, more specific, quantifiable and reproducible, but its sensitivity is low.

(2) RED is used to determine changes in the red system. One of the most frequent and easily observed clinical features in patients with MDS is anemia, which may be related to abnormal erythropoiesis. Mathis et al. proposed a RED score by using nuclear staining to select erythrocytes throughout a non-lysed bone marrow strategy to determine the markers of abnormal erythropoietic-related antigens associated with the diagnosis of MDS. The combination of the two resulted in a combined sensitivity of 87.9% and specificity of 88.9%, sometimes reaching over 90% sensitivity and specificity [16].

In addition, Prof. Zhai et al. refined the flow-set gate scheme and developed the MFCM-Score based on a four-parameter scoring method using CD19 and CD33 to isolate B lymphocyte progenitor cells and myeloid primitive cells in the CD34+ CD45dimm population. This score is simple, convenient, low-cost, and superior to the FCM score in the accurate diagnosis of MDS [17].

3.2.4. Cytogenetics and molecular biology

With the wide application of next-generation sequencing technology, researchers have found that at least 111 genetic mutations in MDS are highly correlated with it. They are mainly involved in nuclear

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DND methylation regulation, histone modifications, RNA spliceosomes, transcription factors and signal transduction pathways. The frequently affected mutations are divided into somatic and germline mutations, including SF3B1, TET2, ASXL1, SRSF2, RUNX1, TP53, U2AF1 and EZH2, among which the mutation frequencies of SF3B1 and TET2 exceed 20%. Although great progress has been made in related studies, the molecular biology diagnosis of MDS still lacks specificity [1, 4]. Only because mutations in SF3B1 gene have specific correlation with myeloid cyclic iron granulocytosis and a high detection rate. In 2016 the WHO revised the diagnostic criteria for MDS-RS accordingly, including genetic mutations in the typing criteria for MDS for the first time [3]. At the 2021 American Society for Hematology (ASH) annual meeting, an investigator explored the effects of SF3B1 gene mutations on the various stages of MDS by methods "Isolating ringed iron-granulated juvenile erythrocytes and detecting SF3B1 genomics" and "SF3B1 mutation in an animal model". The final results showed the importance of SF3B1 somatic gene mutations in the pathogenesis of MDS [18]. The International Working Group for the Prognosis of Myelodysplastic Syndromes (IWG-PM) recommended MDS with SF3B1 mutation (MDS ith mutated SF3B1) as a distinct subtype. This subtype would be the first mutation-based subtype of MDS [19], and it was suggested that the diagnostic criteria for this subtype would be (1) abnormal development of a single red line or multiple lines. (2) Mono- or multilineage hematocrit. (3) SF3B1 somatic gene mutation. (4) Primitive cell bone marrow < 5% and peripheral blood < 1%. (5) Does not meet the 2016 WHO diagnostic criteria for MDS with simple del(5q), MDS/MPN-RS-T or other MDS/MPN, primary myelofibrosis or other myeloproliferative neoplasms. (6) Any karyotype [except del(5q), -7, inv(3) or 3q26 abnormalities and complex chromosomes (≥3)]. (7) Any other somatic gene mutation (except RUNX1 and EZH2) [9]. This subtype often has a good prognosis with a low risk of transformation to leukemia. The significance of abnormalities in other genes in clinical diagnostic staging is not yet known.

Meanwhile, next-generation sequencing technologies answer the question of genetically susceptible myeloid neoplasms. MDS presenting in the elderly is usually associated with age-related somatic mutations, whereas MDS presenting in children and adolescents is more often associated with germline genetic susceptibility [20]. The recognition of familial myeloid neoplasms was highlighted in the 2016 WHO revision, which proposed an increased risk of hematological abnormalities in individuals with specific familial genetic mutations. This recognition was achieved by screening family members of patients with confirmed myeloid neoplasms for early diagnosis and early treatment [3].

3.3. Genomic information-based MDS typing system

Matteo Bersanelli et al. defined a new classification of MDS based on genomic information and designed an individualized prognostic assessment system combining clinical parameters and genomic information. A total of eight different subgroups were identified based on specific genomic features: (1) genomic alterations without specificity [group 0]. (2) SF3B1 mutation + ASXL1/RUNX1 mutation [group 1]. (3) TP53 mutation and/or complex karyotype [group 2]. (4) SRSF2 mutation + TET2 [group 3]. (5) U2AF1 mutation + chromosome 20q-/chromosome 7 abnormality [group 4]. (6) SRSF2 mutation + ASXL1/RUNX1/IDH2/EZH2 mutation [group 5]. (7) SF3B1 mutation alone [group 6]. (8) AML-like mutations [DNMT3A, NPM1, FLT3, IDH1, and RUNX1] [group 7]. Among these 8 genetic subgroups, the main genomic feature in 5 subgroups is splice gene mutations (SF3B1, SRSF2 and U2AF1). Mutations occur early in the disease and these mutations are important in determining the phenotype of MDS, manifesting as different morphological features and survival differences. The prognosis varies considerably between subgroups, with the SF3B1 mutation subgroup having a better prognosis and the TP53 mutation and/or complex karyotype and AML-like mutations having the worst prognosis [21].

This staging system was proposed based on European MDS patients and validated by domestic investigators, and may not be applicable for all Chinese MDS patients. Therefore, we still need to develop a new global universal MDS staging system to better guide the accurate diagnosis and individualized treatment of MDS.

In summary, the WHO has proposed the latest diagnostic typing criteria for MDS based on cytomorphology, immunology, cytogenetics and molecular biology, highlighting the features of abnormal blood cell development and cytogenetic alterations. However, the overall definition of next-generation sequencing technology and molecular biology is relatively superficial, and only SF3B1 gene mutations are included in the diagnostic typing criteria for MDS. With the development of next-generation sequencing technology, more and more data have shown that many genetic test results correlate with MDS staging, and cytogenetic and molecular biology alterations are gaining weight in the diagnostic staging of MDS. The next WHO revision of the MDS diagnostic staging criteria is likely to be a major change and will most likely focus on the molecular biological aspects of NGS. This will

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greatly enrich our understanding of the pathogenesis of MDS and facilitate accurate clinical diagnosis. At the same time, it will be particularly important for the prognosis of patients and the selection of appropriate targeted drugs and other individualized treatments, opening a new era of true precision medicine.

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