Original Article

RING SIDEROBLASTS IN MYELODYSPLASTIC SYNDROMES: MORPHOLOGICAL ASSESSMENT AND DIAGNOSTIC IMPLICATIONS

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ABSTRACT

Introduction. Myelodysplastic syndromes (MDS) are heterogeneous myeloid neoplasms in which accurate identification of ring sideroblasts is essential for classification and prognosis. Perls' staining remains the standard method for demonstrating mitochondrial iron deposition in erythroid precursors. This study evaluates the diagnostic role of this technique in a single-center MDS cohort.

Methods. Forty-four patients evaluated at the University Hospital Center "Mother Teresa" between January 2022 and October 2023 were included. Bone marrow aspirates were examined using Romanowsky–Giemsa and Perls' staining. Dysplasia by lineage, blast percentages, presence of ring sideroblasts, and demographic features were recorded.

Results. Erythroid dysplasia predominated (68%), followed by granulocytic (27%) and megakaryocytic (5%) involvement. Three cases (7%) exhibited ≥15% ring sideroblasts, including one overlap MDS/MPN case with thrombocytosis. Females represented 66% of patients, and most were older than 60 years.

Conclusions. Perls' staining is indispensable for the objective detection of ring sideroblasts and accurate MDS subclassification. Combining morphological assessment with standardized iron staining improves diagnostic precision, particularly in resource-limited settings.

Keywords. Myelodysplastic syndromes; ring sideroblasts; Perls stain; bone marrow; dysplasia; MDS/MPN.

SIDEROBLASTET UNAZORE NË SINDROMET MIELODISPLAZIKE: VLERËSIMI MORFOLOGJIK DHE IMPLIKIMET DIAGNOSTIKE

ABSTRAKT

Hyrje. Sindromat mielodisplazike (MDS) janë neoplazi mieloide heterogjene ku identifikimi korrekt i sideroblasteve unazore ka rëndësi thelbësore për klasifikim dhe prognozë. Ngjyrimi Perls mbetet metoda standarde për demonstrimin e depozitimit mitokondrial të hekurit në

pararendësit eritroidë. Ky studim synon të vlerësojë rolin diagnostik të këtij ngjyrimi në një seri pacientësh me MDS.

Metoda. Në studim u përfshinë 44 pacientë të paraqitur në Qendrën Spitalore Universitare "Nënë Tereza" gjatë periudhës janar 2022–tetor 2023. Aspirimet nga palca e kockës u analizuan me ngjyrime rutinë Romanowsky - Giemsa dhe me ngjyrimin Perls. U vlerësua displazia sipas linjave qelizore, përqindja e blasteve, prania e sideroblasteve unazore dhe karakteristikat demografike.

Rezultate. Displazia u identifikua më shpesh në linjën eritroide (68%), e ndjekur nga linja granulocitare (27%) dhe ajo megakariocitare (5%). Në tre raste (7%) u evidentuan ≥15% sideroblaste unazore, përfshirë një rast me fenotip mbivendosës MDS/MPN me trombocitozë. Predominonte gjinia femërore (66%), ndërsa shumica e pacientëve ishin mbi 60 vjeç.

Përfundime. Ngjyrimi Perls është thelbësor për identifikimin objektiv të sideroblasteve unazore dhe për klasifikimin korrekt të MDS. Kombinimi i vlerësimit morfologjik me kritere të standardizuara për hekurin mitokondrial përmirëson saktësinë diagnostike, veçanërisht në mjedise me burime të kufizuara.

Fjalë kyçe. Sindromet mielodisplazike; sideroblaste unazore; ngjyrimi Perls; palca e kockës; displazi qelizore; MDS/MPN.

INTRODUCTION

Myelodysplastic syndromes (MDS) are clonal myeloid neoplasms characterized by ineffective hematopoiesis, morphologic dysplasia in one or more hematopoietic lineages, and a variable risk of progression to acute myeloid leukemia (AML) (1,2). Clinical presentation is dominated by peripheral cytopenias, particularly anemia, which often follows a chronic, progressive course (1,3).

Biological heterogeneity and morphologic overlap with other hematologic disorders make diagnosis complex.

Molecular studies have shown that MDS arises from hematopoietic stem cells, with progressive accumulation of somatic mutations and cytogenetic abnormalities that significantly influence prognosis and classification (5,6). Despite genomic advances, morphological evaluation of the bone marrow remains fundamental for initial diagnosis (7). Dyserythropoiesis, dysgranulopoiesis, and dysmegakaryopoiesis are the principal morphological features, while the presence of dysplasia in ≥10% of cells within a lineage and the proportion of blasts are key parameters for disease categorization (4,9).

An important subgroup of MDS includes entities with ring sideroblasts, frequently associated with mutations in SF3B1, which carry distinct diagnostic and prognostic implications (10,12,13).

Accurate identification of ring sideroblasts in the bone marrow through Perls' histochemical staining represents an objective and essential criterion for correct classification of these cases.

In this context, the present study aims to analyze bone marrow aspirates for the detection of ring sideroblasts, to evaluate the diagnostic value of Perls' staining, and to compare local experience with international data, highlighting the importance of morphology as the first step in the diagnostic algorithm of myelodysplastic syndromes.

METHODS

Forty-four patients presenting to the University Hospital Center "Mother Teresa" between January 2022 and October 2023 were included in the study. For each case, bone marrow aspirates were examined using routine staining as well as Perls' histochemical staining for the identification of ring sideroblasts. The study had a combined retrospective and prospective design. The number of hematopoietic lineages showing dysplasia, the proportion of blasts, the presence of ring sideroblasts, and patient age and sex were recorded.

Bone marrow aspiration was performed from the posterior iliac crest using 10–20 mL plastic syringes, and the first aspirated fraction (0.5 mL) was used for immediate smear preparation at the bedside. Peripheral blood samples were collected in EDTA tubes in accordance with the recommendations of the International Council for Standardization in Haematology (ICSH). For each patient, at least six bone marrow smears were prepared immediately after aspiration. Two air-dried smears were fixed in fresh methanol and stained with Romanowsky–Giemsa for routine morphological evaluation, while one smear was stained with Prussian Blue (Perls' reaction) to demonstrate mitochondrial iron deposition.

A specimen with known increased iron deposition was used as a positive control. Ring sideroblasts were defined as erythroblasts containing five or more siderotic granules arranged in a perinuclear ring surrounding at least one third of the nuclear circumference; at least 100 erythroblasts were evaluated in each case to determine the percentage of ring sideroblasts. For Perls' staining, smears were fixed in methanol for 5 minutes, treated for 15 minutes with a freshly prepared solution consisting of equal parts 2% potassium ferrocyanide and 0.1 N hydrochloric acid, rinsed with distilled water, counterstained with safranin (1:50 dilution) for approximately 90 seconds, and then air-dried.

RESULTS

Bone marrow aspirates revealed prominent dysplastic changes of the erythroid lineage, including normoblasts with nuclear fragmentation and cells with a markedly megapolychromatophilic appearance. Internuclear bridging between normoblasts was also observed. Dysplastic features of the granulocytic series, characterized by hypogranulation in neutrophils and myelocytes were identified in several cases.

Among the 44 bone marrow smears stained with Prussian Blue (Perls' reaction), three cases showed ≥15% ring sideroblasts (Fig. 1). In these cases, erythroblasts contained more than

five siderotic granules arranged in a perinuclear ring, surrounding more than one-third of the nuclear circumference.

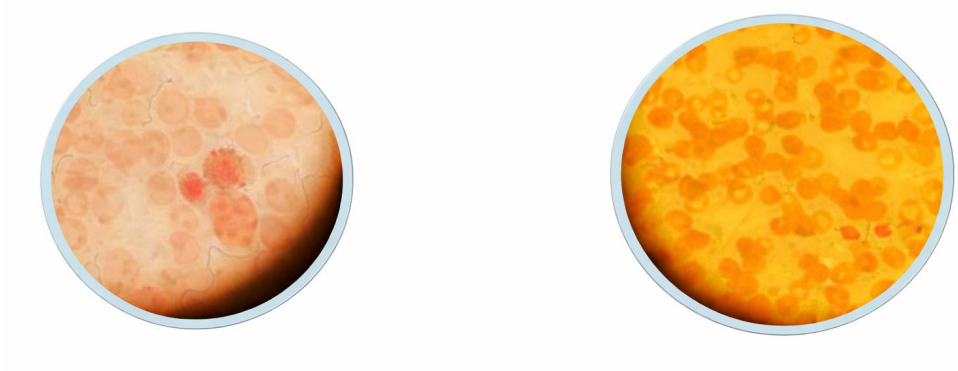

Figure 1. Microscopic appearance of sideroblasts in bone marrow aspirate stained for iron deposits.

The left panel shows non-pathological sideroblasts with minimal cytoplasmic iron granules, whereas the right panel demonstrates sideroblasts with marked perinuclear iron accumulation, characteristic of sideroblastic anemia.

Based on the pattern of cellular dysplasia in patients with MDS and ring sideroblasts, one case exhibited single-lineage dysplasia, whereas two cases demonstrated multilineage dysplasia (Fig. 2).

Figure 2. MDS-RS according to cellular dysplasia.
Distribution of cases with myelodysplastic syndromes with ring sideroblasts (MDS-RS) according to the pattern of cellular dysplasia (single-lineage versus multilineage involvement).

One patient fulfilled criteria for an overlap myelodysplastic/myeloproliferative neoplasm (MDS/MPN), presenting with thrombocytosis (>450,000/mm³), erythroid dysplasia (internuclear bridging, megaloblastoid changes, and multilobulated nuclei), increased

numbers of monolobulated megakaryocytes, absence of granulocytic dysplasia, and <1% blasts in the bone marrow. Perls' staining confirmed ≥15% ring sideroblasts within the erythroid lineage, consistent with the phenotype of MDS/MPN with ring sideroblasts and thrombocytosis.

In the demographic analysis, of the 44 patients included, 15 were male (34%) and 29 female (66%). The youngest patient was 32 years old and the oldest 85 years. Most patients belonged to the 60–69-year age group (45.5%), followed by those aged 50–59 years (29.5%) (Table 1, Fig. 3). When stratified by a 60-year age cut-off, females predominated in the <60-year group, whereas sex distribution was more balanced among patients aged ≥60 years (Table 2).

Table 1. Age distribution of patients with myelodysplastic syndromes (N = 44)
The minimum age was 32 years, and the maximum age was 85 years.

Age group (years)	**Number of patients (n)**	**Percentage (%)**
30–39	1	2.3
40–49	1	2.3
50–59	13	29.5
60–69	20	45.5
70–79	6	13.6
80–89	3	6.8
Total	**44**	**100**

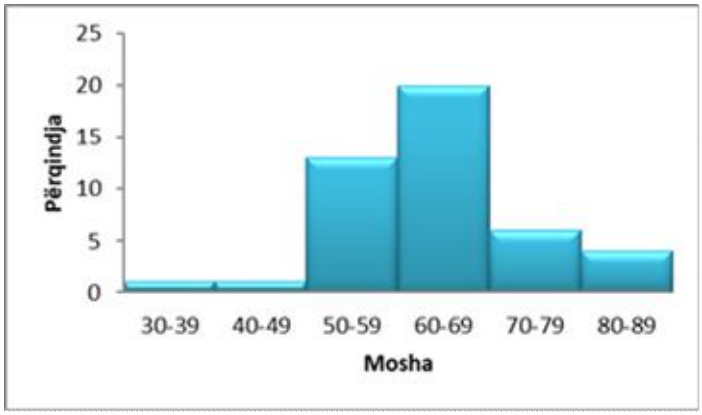

Figure 3. Age distribution of patients with myelodysplastic syndromes included in the study. *Histogram showing the percentage of patients across age groups, with the highest frequency observed in the 60–69-year category.*

Table 2. Sex distribution of patients with myelodysplastic syndromes according to age group (N = 44)

Age group	**Male, n**	**Female, n**	**Total**
<60 years	4	16	20
≥60 years	11	14	25*
Total	**15**	**29**	**44**

Assessment of dysplasia across hematopoietic lineages showed that the erythroid series was most frequently affected (68%), followed by the granulocytic lineage (27%) and the megakaryocytic lineage (5%).

DISCUSSION

In our study, MDS with ring sideroblasts accounted for approximately 7% of all myelodysplastic syndromes, a proportion consistent with international reports that place this subtype at 5-10% of MDS cases. Moreover, cases of MDS with single-lineage dysplasia (MDS-SLD) and ring sideroblasts were less frequent than those with multilineage dysplasia (MDS-MLD), in agreement with large European retrospective series (15).

The overlap entity MDS/MPN with ring sideroblasts and thrombocytosis (MDS-RS-T) was identified in only one patient, reflecting the rarity of this diagnosis in routine clinical practice. This observation aligns with data reported by Patnaik and colleagues (16), who described a relatively small cohort of patients with this phenotype and a median age in the seventh decade of life.

Demographic analysis showed a predominance of female patients under age 60, a finding also reported in the Polish national MDS registry (11). However, this pattern contrasts with several other European cohorts, which report a slight male predominance, including data from the United Kingdom and Romania. Such discrepancies may reflect geographic variability, environmental influences, differences in population structure, or methodological heterogeneity among national registries (4,8,14,15).

Consistent with previous reports, most patients in our cohort were older than 60 years, confirming that MDS remains predominantly a disease of advanced age. Similar age distributions have been reported in studies from Italy, Germany, Austria, and North Africa,

where the median age generally ranges from 65 to 70 years and the proportion of patients younger than 50 years is relatively low (4,9).

Case classification in our series relied primarily on bone marrow morphologic assessment, which showed a predominance of erythroid dysplasia. This approach mirrors routine practice in many diagnostic laboratories, particularly in settings where cytogenetic and molecular analyses are not consistently available. As emphasized in international guidelines, morphology is the initial step in the diagnostic algorithm for MDS, whereas Perls' staining remains essential for the objective identification of ring sideroblasts and for accurate disease classification (9,17,18).

Nevertheless, several limitations should be considered when interpreting our findings. The relatively small sample size limits statistical power and the generalizability of the results. In addition, variability in smear quality may have influenced morphological evaluation, and the absence of cytogenetic and molecular testing precluded comprehensive classification according to contemporary WHO and consensus systems. Future studies incorporating larger patient cohorts and integrated morphological, cytogenetic, and molecular data will be necessary to validate these observations and to refine diagnostic and prognostic stratification in patients with MDS and ring sideroblasts.

CONCLUSION

Because dysplastic changes are primarily assessed morphologically, their interpretation may be subjective. In our cohort, systematic use of Perls' staining enabled objective identification of ring sideroblasts using quantitative criteria and proved essential for accurate subclassification of myelodysplastic syndromes. The detection of cases with both single-lineage and multilineage dysplasia, as well as an overlap MDS/MPN phenotype, underscores the diagnostic value of this technique in routine practice.

Our findings support the role of Perls' staining as a critical component of the diagnostic algorithm for MDS, contributing not only to disease classification but also to staging and prognostic assessment. In settings with limited access to cytogenetic and molecular testing, careful morphological evaluation combined with standardized iron staining remains indispensable for guiding clinical decision-making and aligning local diagnostic practice with international standards.

Conflict of interests. The authors declares no conflict of interests.

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