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Spectrum of Morphologic Findings in Myelodysplastic Syndrome with Ring Sideroblasts

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Abstract: Background Myelodysplastic syndrome with ring sideroblasts (MDS-RS) is a subtype of myelodysplastic syndrome (MDS) according to the WHO 2016 classification. Although IWGM-MDS recommended the defination of ring sideroblasts, most of the clinicians in China counting the ring sideroblasts were still depending on their own standard which was based on experience. Varying definitions of ring sideroblasts led to confusion among clinicians and misdiagnosis of MDS-RS. Methods In this article, we proposed the new method for solving this question by reviewing 26 MDS-RS cases about their clinical, laboratorial, morphological and molecular findings retrospectively. Results The analysis of our data showed that accompanying the MDS-RS were severe erythroid dysplasia or mild erythroid megaloblastic changes. It was a key role that ring sideroblasts played in diagnosing MDS-RS. Therefore we recommended iron stain be performed routinely for anemia patients undergoing bone marrow aspiration to exclude MDS-RS. It was important to take both quantity and size of granules into account in assessing ring sideroblasts. By analyzing ring sideroblasts in our cases we redefined two types of the ring sideroblasts. The type1 was more than 11 granules either distributed into rings or covering two third of the perinuclear area approximately. The type 2 was 6-10 granules covered more than one third of the perinuclear area, the granules must be coarse enough to cover at least one third of the perinuclear area when having closely connected. Conclusion In summary, our results were helpful for Chinese clinicians to master the morphologic features easily, unify the standrad of the ring sideroblasts accurately and avoid the misdiagnosis of MDS-RS effectively.

Keywords: Myelodysplastic syndrome with ring sideroblasts; Ring sideroblasts; Erythroid dysplasia; Anemia; Iron stain

1. Introduction

Recent researches showed SF3B1 is highly mutated in MDS-RS and induced the formation of ring sideroblasts.[1-4] According to the 2016 revision to the WHO classification of myeloid neoplasms and acute leukemia, the category of MDS-RS were including at least 15% ring sideroblasts in cases lacking SF3B1 mutations or 5% ring sideroblasts in cases with SF3B1 mutations and single lineage(classified as RARS previously) or multilineage dysplasia(classified as RCMD previously), refractory anemia or cytopenia and bone marrow(BM) indicating morphologically abnormal.[5-8] Cases with isolated del (5q) and excess blasts were excluded.[9]
Non—neoplastic causes of ring sideroblasts, including toxins, drugs, alcohol, copper deficiency, and congenital sideroblastic anaemia must be excluded. Owing to the absence of biological markers for patients with MDS-RS, the main method to diagnose the MDS-RS was morphological assessment especially count of ring sideroblasts. IWGM-MDS recommended that ring sideroblasts be defined as erythroblasts in which there are a minimum of five siderotic granules covering at least one third of the circumference of the nucleus.[10] However, there is still confusion and controversy in MDS-RS diagnosis among clinicians in China. Most of them judge and count ring sideroblasts based on their experience because maybe it is difficult for them to comprehend the defination of ring sideroblasts which seems unfit for their MDS-RS patients. Here, for the first time, we have observed 26 MDS-RS cases in our files to reveal some interesting findings.

2. Materials and Methods

Our study has received ethics committee approval. We reviewed our files for cases that met the WHO classifica-

tion 2016 criteria for MDS-RS. 26 confirmed MDS-RS cases were enrolled in our study. The patients data were collected including age, gender, WBC count , platelet count, hemoglobin level, ring sideroblasts count and BM morphologic features. BM aspirate smears with Wright-Giemsa stain were analyzed. Iron stain with Prussian blue was done on BM smears, and count of ring sideroblasts in 100 nucleated erythroblasts was performed to quantify ring sideroblasts. Ring sideroblasts were counted according to the 2008 WHO classification of Tumours of Haematopoietic and Lymphoid Tissues.[11] Available cases was performed conventional cytogenetics analysis on BM aspirate material. SF3B1 mutations were analyzed using genomic sequencing method for available cases.

3. Clinical and Laboratorial Findings

26 MDS-RS patients including 13 men and 13 women were enrolled in our researches, the age of the patients range from 39 to 92 years old, with a median age of 67.85 years old.(Table1) 16 patients presented with fatigue and weakness symptoms at first visit to see a doctor, 1 had ecchymosis in skin, 1 occured weight loss and pale skin, 2 reported flu-like symptoms, and 6 patients had no apparent symptoms, abnormal results of blood routine examination were detected incidentally. Physical examination findings were no significant abnormity in our cases. Laboratorial findings showed that all patients had varying degrees of anemia at the time of diagnosis. The hemoglobin level ranged from 44 to 90 g/L, with a median of 72.31 g/L(reference range, 115-150 g/L for women and 130-175 g/L for men). The platelet count ranged from 22 to 603 $\times 10^9$/L with a median of 192.58 $\times 10^9$/L(reference range, 125-350 $\times 10^9$/L), the platelet count was 125 $\times 10^9$/L lower in 11 patients and 350 $\times 10^9$/L higher in 4 patient. WBC count ranged from 1.8 to 17.6$\times 10^9$/L with a median of 4.88 $\times 10^9$/L(reference range:3.5-9.5 $\times 10^9$/L), WBC count was 3.5 $\times 10^9$/L lower in 7 patients and 9.5 $\times 10^9$/L higher in 2 patient.

Table 1. Features of 26 Cases of Myelodysplastic Syndrome with Ring Sideroblasts

Case No./Sex/ Age (y)	WBC ($\times 10^9$/L)	PLT ($\times 10^9$/L)	HB (g/L)	RS (%)	erythroid dysplasia	Granulocyte dysplasia	Megakaryocytic dysplasia
1/F/66	4.4	202	71	32	Y	N	N
2/F/66	11.8	110	68	35	Y	N	N
3/M/39	8.7	511	89	20	Y	N	N
4/F/66	4.3	377	79	32	Y	N	N
5/M/70	5.2	211	88	60	Y	N	N
6/F/72	2.5	85	89	25	Y	Y	Y
7/F/88	3.9	131	78	28	Y	N	N
8/F/61	2.2	143	90	20	Y	N	N
9/F/70	2.7	237	88	44	N	N	N
10/M/92	17.6	603	51	36	Y	N	N
11/F/62	5.8	122	77	20	N	N	N
12/M/56	1.8	39	75	18	Y	Y	Y
13/M/81	2.4	174	63	44	Y	Y	N
14/M/74	4.3	337	60	17	N	N	N
15/M/81	5.1	126	72	18	Y	N	N
16/F/72	3.6	89	67	29	Y	Y	Y
17/F/64	5.0	70	64	35	Y	Y	N
18/M/61	3.7	115	72	30	Y	N	N
19/M/71	4.9	95	79	17	Y	N	Y
20/F/79	3.7	139	81	25	Y	Y	N
21/M/47	4.0	193	68	62	Y	N	N
22/M/70	3.8	577	51	10	Y	Y	Y
23/M/57	2.3	41	58	15	Y	N	N
24/F/49	3.1	22	46	16	Y	N	N
25/M/73	6.3	123	89	18	Y	N	N
26/F/77	3.9	135	67	60	Y	N	N

※the threshold to define dysplasia is 10% dysplastic cells in any hematopoietic lineage.RS, ring sideroblasts.

4. Morphologic Findings

Peripheral blood smears of 20 cases were available to review. Anemia was present in all these cases with macrocytic normochromic in 15 cases and normocytic normochromic in 5 cases. Blood smears showed anemia was present as normochromic with red cells varied in size and red blood cells fragments. [Figure 1a] Peripheral blood smears of 4 cases showed neutrophils dysplastic features including pseudo-Pelger-Huët nuclear anomaly and absence of cytoplasmic granulation in segmented granulocytes. 3 cases showed decreased platelets with platelets varied in size. BM smear showed blasts proportion were

fewer than 5% and erythroid precursors proportion were increased in all cases.

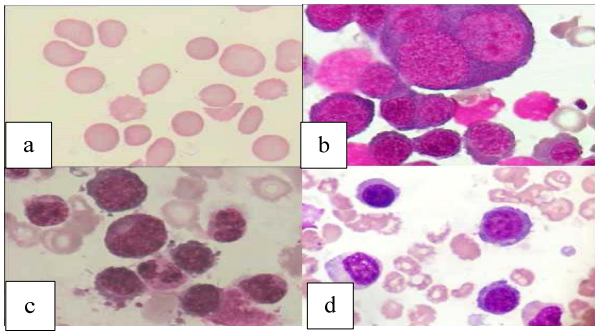
In our study, we found an interesting phenomenon. Almost all of the *previous* studies supported all MDS-RS patients were characterized by isolated erythroid dysplasia or multilineage dysplasia and more ring sideroblasts in BM cell morphology. However, in our study, 20 patients had severe erythroid dysplasia with higher proportion(≥10%), including binuclear and multinuclear of varying sizes and imbalance development, abnormal mitosis, malformed nucleus and lobate nucleus with fine bridge, etc;[Figure 1b] 3 patients had obvious condensation of nuclear chromatin and ragged cytoplasm with ill-defined edges with higher proportion(≥10%); [Figure 1c]The rest 3 patients had no obvious erythroid dysplasia but mild erythroid megaloblastic changes, similar to atypical megaloblastic anemia.[Figure 1d] 7 patients had granulocyte dysplasia including pseudo-Pelger-Huët nuclear anomaly and cytoplasmic hypogranulation with higher proportion(≥10%). 5 patients had megakaryocyti dysplasia including hypolobated or bilobed nuclei, multiple separated nuclei, and micromegakaryocytes with higher proportion(≥10%). In our cases, 3 patients had no obvious abnormal dysplasia in erythron series, granulocyte series and megakaryocytic series except mild erythroid megaloblastic changes and more ring sideroblasts(≥15%), and they all harbour SF3B1 mutations. These patients would be misdiagnosed with another illness without accurate count of ring sideroblasts. Therefore, it's important that iron stain should be performed routinely for anemia patients undergoing bone marrow aspiration to exclude MDS-RS. Count ring sideroblasts correctly is vital for diagnosis of MDS-RS.

5. Findings about Count of Ring Sideroblasts

The IWGM-MDS proposed the definition of a ring sideroblast is an erythroblast with at least 5 siderotic granules covering at least a third of the circumference of the nucleus, and this has been incorporated into the 2008 WHO classification of Tumours of Haematopoietic and Lymphoid Tissues. In our studies, ring sideroblasts according to this standard were clasified into three types. Type 1 was at least 11 siderotic granules distributed into rings or covering two third of the perinuclear area approximately. Type 2 was siderotic granules between 6 and 10 covering at least a third of the circumference of the nucleus. Type 3 was 5 siderotic granules covering at least a third of the circumference of the nucleus . In all our cases, type1 ring sideroblast ranged from 9 to 58 with a median of 25.5, type2 ranged from 1 to 6 with a median of 4.0 and we didn't find type 3 ring sideroblast in all our cases. We discovered when 5 siderotic granules closely connected they couldn't cover one third of the perinuclear area, only a few 6-10 siderotic granules met covering one third of the perinuclear area approximately when the siderotic granules were thick and coarse enough. In our researches, the ring sideroblasts of all the patients were more than 15% except that the ring sideroblasts of a patient with SF3B1 mutations was 10%. The vast majority of ring sideroblasts(86.4%) belonged to type1.[Figure 2a]Another interesting phenomenon was observed when BM smears after Prussian blue staining were reviewed. Ring sideroblasts in a MDS-RS patient showed that there was blank area between nucleus of erythroid precursors and ring consisting of siderotic granules.[Figure 2b] It was not clear about mechanism of the blank area appearance. Perhaps it was interactions between the antibody in erythrblasts cytoplasm and Prussian blue dye that made the phenomenon.

6. Molecular Findings

3 cases were available to do cytogenetic analysis and all revealed a normal diploid karyotype. We analyzed SF3B1 mutations using genomic sequencing method for available cases. SF3B1 mutations were analyzed in 20 cases, and SF3B1 mutations in 13 patients occured. We didn't find SF3B1 mutations to be correlated with the degree of anemia and erythroid dysplasia.



a: Blood smear showed anemia was present as normochromic with red cells varied in size and red blood cells fragments ;

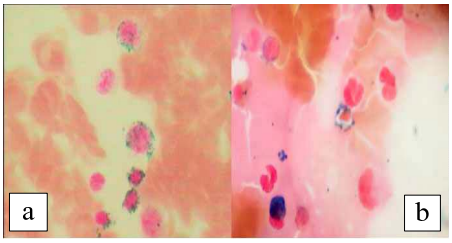
BM smear showed

b: severe erythroid dysplasia including binuclear and multinuclear of varying sizes and imbalance development in MDS-RS.

c: obvious erythroid dysplasia including condensation of nuclear chromatin and ragged cytoplasm with ill-defined edges in MDS-RS.

d: no obvious erythroid dysplasia but mild erythroid megaloblastic changes, similar to atypical megaloblastic anemia in MDS-RS.

(Wright-Giemsa, ×1000)



a: Bone marrow smear after Prussian blue staining showed that most of the erythroid precursors were ring sideroblasts with at least 11 siderotic granules distributed into rings or covering two third of the perinuclear area approximately (prussian blue staining, x1000)

b: Ring sideroblasts in a MDS-RS patient showed there was blank area between nucleus of erythroid precursors and ring consisting of siderotic granules. (prussian blue staining, x1000)

7. Discussion

One of the most common subtypes of MDS is MDS-RS. The disease is characterized by ring sideroblast, defined as at least 15% ring sideroblasts in cases lacking SF3B1 mutations or only 5% ring sideroblasts in cases with SF3B1 mutations and single lineage or multilineage dysplasia, and refractory anemia or cytopenia.[12-13] The purpose of this paper was to retrospectively analyze the cases in our files that met WHO 2016 classification criteria for MDS-RS to summarize clinical and morphologic features, redefine and unify the standard of ring sideroblasts accurately and avoid misdiagnosis of MDS-RS in China.

As we all known, morphological assessment is vital to dignose the disease and ring sideroblasts play a key role in the MDS-RS dignosis. It's vital that iron stain should be performed routinely for anemia patients undergoing bone marrow aspiration to exclude MDS-RS.The reasons are as follows: First, the 2016 WHO classification relies primarily on the degree of dysplasia and blast count, and specific cytopenias have been a minor factor on MDS diagnosis; Second, One of the main challenges in MDS diagnosis is separating MDS from reactive reasons of dysplasia and cytopenias. Although the threshold of dysplasia definition is 10% dysplasia in any hematopoietic lineage, it is comfirmed that dysplasia more than 10% may happen in some normal individuals. Moreover, even among experienced clinicians it is not always reproducible in assessment of dysplasia; Finally, although all patients presented with varying degrees of anemia, our study indicated that most of the MDS-RS patients had severe erythroid dysplasia, and a few had no obvious erythroid dysplasia but mild erythroid megaloblastic changes. In our cases, 3 patients had no obvious abnormal dysplasia in erythron series, granulocyte series and megakaryocytic series except mild erythroid megaloblastic changes and more ring sideroblasts. In a word, count of ring sideroblasts accurately is important for diagnosis of MDS-RS.

While the iron of ferritin sideroblasts is encoded by the FTH1 and FTL genes, the iron of ring sideroblasts is encoded by the FTMT gene.[14] The former storing in cytosolic ferritin of normal BM shows a few blue granules scattered in the cytoplasm, the latter is stored in mitochondrial ferritin and erythroblasts with mitochondrial iron overloading lead to abnormalities of mitochondrial function and formation of ring sideroblasts. Moreover, some researchers indicated iron overload is an important problem in the pathogenesis, morbidity and prognosis of patients with MDS-RS.[15-16] If clinicians are confused with ring sideroblasts and ferritin sideroblasts, MDS-RS would be misdiagnosed. Thus severely affect treatment effect and the prognosis of the patients. However, most of the clinicians from different hospitals in China realized the standard of ring sideroblasts defined by IWGM-MDS was difficult for them to comprehend and seemed unfit for their MDS-RS patients. They have their own standard to count ring sideroblasts based on their experience to this day. Varying definitions of ring sideroblasts resulted in confusion among clinicians and misdiagnosis of MDS-RS. In our studies, we emphasized judgement of ring sideroblasts which characteristic is coarse iron deposits in mitochondria should take both quantity and size of granules into account. The vast majority of ring sideroblasts(86.4%) belonged to type 1 defined by us as at least 11 granules either distributed into rings or covering two third of the perinuclear area approximately. When 6-10 granules distributed the perinuclear area, granules of ring sideroblasts must be coarse enough to cover more than one third of the perinuclear area when having closely connected.

There are frequent occuring somatic mutations in splicing, transcription and epigenetic factors such as TET2, ASXL1, RUNX1, SRSF2, and SF3B1 or chromosomal abnormities, including monosomy 7 and 5q deletions in MDS patients.[17] Although some researchers have identified that SF3B1 mutations occured in 60%-80% of patients with RARS and RARS-T (RARS associated with thrombocytosis) patients, until now the clonal nature of MDS-RS has been questioned.[18] Valeria Visconte et al indicated SF3B1 mutants changed iron distribution characterized by coarse iron deposits in mitochondrial in comparison with wild-type RARS patients. It has been reported by them there was experimental evidence of the association between SF3B1 and ring sideroblasts existence and they suggested that SF3B1 mutations led to ring sideroblasts formation. Moreover, SF3B1 mutation represented a favourable prognosis value for MDS-RS. In our studies, SF3B1 mutations were analyzed in 20 cases, and SF3B1 mutations in 13 patients occured. In summary, our studies offered a new strategy to comprehend diagno-

sis of MDS-RS and the redefinition of ring sideroblasts. Our results recommended iron stain be performed routinely for anemia patients undergoing bone marrow aspiration to exclude MDS-RS, and both quantity and size of granules be taken into account in judging ring sideroblasts.

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