



Transient/reversible ring sideroblasts in bone marrow of patients post cytotoxic therapies for primary malignancies

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ABSTRACT

The diagnosis of therapy-related myelodysplastic syndrome (t-MDS) in the absence of increased myeloblasts or cytogenetic abnormalities is challenging. The presence of ring sideroblasts (RS) in this setting is often used to support the diagnosis of t-MDS. In this study, we reviewed 843 patients initially classified as therapy-related myeloid neoplasm in our hospital over 10 years. Nineteen (2.3%) patients had a normal karyotype, <5% bone marrow (BM) blasts, and $\geq 15\%$ RS (17–70%), forming this study group. After reviewing clinical charts and follow-up BM specimens, we confirmed the diagnosis of MDS in 13 patients, but in 6 patients the blood counts returned to normal and RS and associated dyserythropoiesis disappeared in the follow-up BM biopsy. With a median follow-up of 21 months, none of these 6 patients died of BM causes. Compared with t-MDS cases, the 6 patients with transient/reversible RS showed comparable numbers of RS and BM blasts, but infrequent dysplasia involving non-erythroid lineages. We conclude that the presence of $\geq 15\%$ RS in the post-therapy setting is not necessarily indicative of a clonal stem cell neoplasm. Four patients with transient/reversible RS received α -interferon therapy which may contribute to RS formation in this setting.

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1. Introduction

Assessment of cytopenia(s) in patients who have a clinical history of cancer treated with various therapeutic agents can be challenging. Cytopenia can be attributed to a number of different causes or be multifactorial, including bone marrow (BM) suppression or maturation arrest secondary to drugs, paraneoplastic syndromes, infections, nutritional deficiencies, liver or renal insufficiency; or increased destruction or loss of blood cells. Therapy-related myeloid neoplasms (t-MN) secondary to prior exposure to chemotherapy or ionizing radiation is an infrequent but serious therapeutic complication. After a review of the patient's medical history and thorough laboratory work-up, BM aspiration and biopsy are frequently performed, either because of an unrevealing laboratory work-up or a compelling reason to exclude t-MN with greater assurance.

According to the current World Health Organization (WHO) classification system, t-MN include acute myeloid leukemia (t-AML), myelodysplastic syndrome (t-MDS) and therapy-related

myelodysplastic/myeloproliferative neoplasms (t-MDS/MPN) [1] t-MN is usually associated with a dismal prognosis and thus reliable diagnosis is essential. Approximately half of t-MDS patients have $\geq 5\%$ of blasts in the BM and >90% of patients with t-MN harbor cytogenetic abnormalities [1] which often helpful in establishing a diagnosis of t-MN. In contrast, morphologic assessment of dysplastic changes is less reliable in the workup of t-MDS as dysplastic changes can be observed in patients undergoing various therapies or in patients with active cancers and their associated complications.

The presence of a substantial number of ring sideroblasts (RS) is one of the morphologic manifestations of dysplasia and is often used to support the diagnosis of t-MN [1,2]. The rationale behind this practice is that RS are a reflection of abnormal iron metabolism that commonly occurs in the MDS setting. Although RS can occur as a result of alcohol ingestion [7,8], toxin exposure (e.g. benzene) [4–6], mineral imbalance (e.g. copper deficiency) [9–11], pregnancy [3,4], congenital disorders [12–15], most of these causes are uncommon in the clinical setting of cancer treatment. Furthermore, most of the medications known to cause RS are antimicrobial (bacteriostatic) drugs, such as isoniazid, pyrazinamide, chloramphenicol, D-penicillamine, cycloserine, fusidic acid, linezolid, tetracycline analogue [5–8] that usually are not ingested by patients at time of BM examination during the MDS workup. The association of transient/reversible RS as a direct result of chemotherapy has only been rarely reported in patients treated with Busulfan [9,10].

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t-MN is a serious condition, which often affects patients' treatment plan for primary cancer and required appropriate management for t-MN, including stem cell transplant. We conducted the current study to assess the use of RS as evidence of t-MDS in patients treated for primary cancer where the BM had less than 5% blasts and normal cytogenetics.

2. Materials and methods

2.1. Study group

The files of the Department of Hematopathology at The University of Texas MD Anderson Cancer Center (MDACC) from 2002 to the present were searched for patients with a diagnosis of t-MN (or MDS) or "consistent with t-MN" or "suggestive of t-MN". Clinical information was obtained by review of the electronic medical records for prior malignancies, detailed treatment information, follow-up BM aspiration and biopsy specimens, complete blood counts (CBC), management changes and patients outcomes. For each case, a CBC with a differential count, BM findings, flow cytometry immunophenotypic data, conventional cytogenetic and fluorescence in situ hybridization (FISH) data, and the results of molecular studies were incorporated. Disease-specific survival (DSS) was calculated from the day of t-MN diagnosis until death from t-MN. This study was approved by the Institutional Review Board (IRB) of MDACC.

2.2. Morphologic evaluation

Bone marrow aspirate smears at the time of t-MDS were available for evaluation in the study group. A 500 cell differential count was performed based on examination of multiple fields of BM aspirate smears. For the assessment of morphologic dysplasia in BM, features of dyserythropoiesis, dysgranulopoiesis, and dysmegakaryopoiesis had to be present $\geq 10\%$ of cells of the respective lineages, as specified in the 2008 WHO classification [1]. Iron stain was performed on the aspirate smears to assess storage iron and RS. A RS was defined as the presence of 5 or more perinuclear dots surrounding at least one third of the nucleus in erythroid precursors according to the criteria proposed by the MDS International Working Group (IWG) [11] and the WHO classification [1]. The cutoff we chose for RS was $\geq 15\%$ of the BM erythroid precursors. The 15% cutoff was chosen to identify cases with significant number of RS; which was in line with the criteria used by the WHO classification to distinguish refractory anemia (RA) from refractory anemia with ring sideroblasts (RARS); and recommendation as one of the dysplastic features by the International Working Group (IWG) for MDS [2].

2.3. Cytogenetic analysis

Conventional cytogenetic analysis was performed using standard methods as previously described [12]. Metaphase cells were obtained from unstimulated BM aspirate cultures after hypotonic treatment and fixation with 3:1 methanol-acetic acid solution. Cell suspensions were dropped onto clean slides. G-banding was performed after the slides were dried at 60°C overnight. Twenty metaphases were analyzed in all cases, if available, and the results reported using the International System for Human Cytogenetic Nomenclature. In some cases, fewer than 20 metaphases were available, but in all cases the number of metaphases was adequate for conventional cytogenetic analysis. FISH was performed selectively in a subset of cases when a case was highly suspected for t-MDS, but no clonal chromosomal abnormalities were detected by conventional cytogenetics.

2.4. Statistical analysis

The Mann-Whitney test was used for numerical comparison between two groups. The Fisher exact and chi-square tests were applied for categorical variables. Differences between groups were considered statistically significant if *p*-values were less than 0.05 in a two-tailed test.

3. Results

3.1. Patients

In a retrospective manner, 843 patients were identified in whom a diagnosis of t-MN, "consistent with t-MN" or "suggestive of t-MN" were identified in our hospital from 2002 to 2010. We then focused on 126 (15%) cases that had a normal karyotype (including loss of chromosome Y) [16]. In this subgroup, 45 patients had t-AML, 2 patients had t-MDS/MPN (CMML), and 79 patients were classified as t-MDS. Within the t-MDS subgroup, 14 patients had $\geq 5\%$ myeloblasts in the BM, and were excluded. Of the remaining

65 cases with $< 5\%$ myeloblasts in the BM, 19 patients had $\geq 15\%$ RS of the erythroid precursors. These patients formed the study group.

After reviewing treatment information, clinical findings, follow-up CBC, follow-up BM aspirate and biopsy specimens, the 19 patients were subdivided into those patients with true MDS and those without MDS. In the former group, 10 patients had t-MDS, secondary to prior cytotoxic therapies. These 10 patients included 7 men and 3 women with a median age of 70 years (range 25–85). Five of the patients had hematolymphoid malignancies and five patients had solid tumors, including prostate cancer, Ewing sarcoma, melanoma, thymoma, and colon cancer. Treatment modalities were highly variable but included combined chemoradiation ($n = 3$), radiation therapy only ($n = 3$) and chemotherapy only (4 patients). Three additional patients fulfilled the diagnostic criteria for MDS; however, BM examination prior to the initiation of cytotoxic therapies had already demonstrated evidence of MDS or the patients had never received cytotoxic therapy for cancer. These cases are likely to be coincidental MDS unrelated to therapy.

The remaining 6 patients had numerous RS in BM that subsequently disappeared in follow-up BM specimens. The cytopenia(s) these patients had also improved without therapeutic intervention, calling into question the diagnosis of t-MDS. The clinicopathologic features of these patients are summarized in Table 1. The detailed treatment information and follow up data for these 6 cases are presented here.

3.2. Patient #1

A 24-year-old woman was diagnosed with metastatic melanoma involving inguinal lymph nodes and underwent high dose interleukin-2 (IL-2) infusion as the initial treatment. She was noticed having mild thrombocytopenia at the end of treatment ($108 \times 10^9/L$). Her melanoma was unresponsive to IL-2 treatment and continued to spread to liver, bones and more lymph nodes. She received biochemotherapy consisting of cisplatin, velban, dacarbazine, IL-2 and α -interferon and became pancytopenic in the course of treatment. After completion of 4 cycles of biochemotherapy, her WBC recovered ($ANC = 4 \times 10^9/L$), but she remained to be anemic ($Hb = 9.1 \text{ g/dL}$, $MCV = 85 \text{ fL}$, $RDW = 15.6$) and thrombocytopenic (platelets = $57 \times 10^9/L$). She was scheduled for weekly Paclitaxel with cisplatin embolization. However, she further dropped her platelets to $19 \times 10^9/L$ after the first dose of Paclitaxel. A BM biopsy was performed 5 days post Paclitaxel, 6 months from initiation and 3 months after completion of biochemotherapy. The BM examination revealed a 50% cellularity, 0% blasts, normal megakaryocytes, orderly myeloid maturation, a near normal myeloid:erythroid ratio (2:1) with 19% RS. There was no evidence of metastatic melanoma. In addition to 19% RS, many sideroblasts were seen and storage iron was markedly increased (4/4). Chromosomal analysis revealed a normal karyotype. FISH was not performed. The BM findings, met the morphologic criteria for RARS. Another BM biopsy was performed 9 days later for confirmation and demonstrated a normal cellularity with trilineage hematopoiesis, and there were no RS or dysplasia. The patient passed away 9 days later due to progression of metastatic melanoma.

3.3. Patient #2

A 54-year-old man with a 30-month history of stage III IgA multiple myeloma (MM) was evaluated by hematology consult service for pancytopenia. His MM was initially treated with thalidomide and dexamethasone and 2000 cGy of radiation to the T1 to L2 vertebral bodies for compression fractures. He achieved a complete response and underwent autologous stem cell transplant after high-dose melphalan conditioning. One year later, he showed

Table 1

Clinicopathologic comparison of patients with therapy-related myelodysplastic syndromes with ring sideroblasts and patients with transient/reversible ring sideroblasts.

	t-MDS (n = 10)	Transient RS (n = 6)	p
Age (years)	71 (range 25–85)	62 (range 24–82)	0.48
Gender (male:female)	7:3	4:2	1.0
Complete blood cell count (CBC)			
Hemoglobin (g/dL)	9.4 (range 7.7–13.6)	10.1 (range 6.8–12.0)	0.95
MCV (fL)	96 (range 85–105)	92 (range 83–106)	0.70
RDW (%)	16.2 (14.9–18.8)	20.9 (13.4–25.8)	0.07
Absolute neutrophil count ($\times 10^9/L$)	2.9 (range 0.3–6.0)	2.5 (range 1.0–4.0)	0.59
Platelet ($\times 10^9/L$)	70 (range 20–314)	100 (range 22–231)	0.56
Bone marrow (BM)			
Cellularity (%)	40 (range 30–75)	45 (range 20–70)	0.96
BM myeloblasts (%)	1 (range 0–4)	1 (range 0–4)	0.73
Erythroid proportion (%)	38 (13–64)	24 (14–40)	0.11
Ring sideroblasts (RS) (%)	30 (17–75)	38 (17–70)	0.83
Dysplasia			0.04
Erythroid only	2	5	
Involving other lineage	7	1	
Follow-up BM biopsy	RS persisted (8/8 cases)	RS disappeared 5/5 cases	
History of cytotoxic therapy			
Radiation + chemotherapy	n = 3	n = 2	
Radiation only	n = 3	n = 1	
Chemotherapy only	n = 4	n = 3	
Interval between detection of RS and prior cytotoxic therapy (months)	63 (14–251)	33 (6–132)	0.39
Survival data	n = 10 (6 dead and 4 alive)	n = 6 (3 dead and 2 alive and 1 lost follow up)	
Died from MDS progression	2	0	
Died from cancer	3	3	
Died of unknown cause	1	0	

signs of recurrence, and treated with α -interferon 4,000,000 units subcutaneously for 6 months, and subsequently with intermittent high dose dexamethasone with Revimid due to disease progression. He developed pancytopenia requiring intermittent Neupogen and Procrit. His CBC showed Hb = 9.7 g/dL, MCV = 83 fL, RDW = 18.1, WBC = $2.1 \times 10^9/L$ with ANC of $1.0 \times 10^9/L$ and platelet count $56 \times 10^9/L$. He received his last dose of Revimid 10 days prior to

this BM evaluation. His BM revealed a variable cellularity (10–70%, overall 20%), 2% blasts, no megakaryocytic or granulocytic dysplasia, mild dyserythropoiesis with 70% RS (Fig. 1A and B). There was no evidence of plasma cell neoplasm. Conventional karyotyping revealed a normal karyotype. He was considered to have RARS by pathologic criteria. However, in order to rule out the possibility of residual Revimid effect, a BM biopsy was repeated 20 days later,

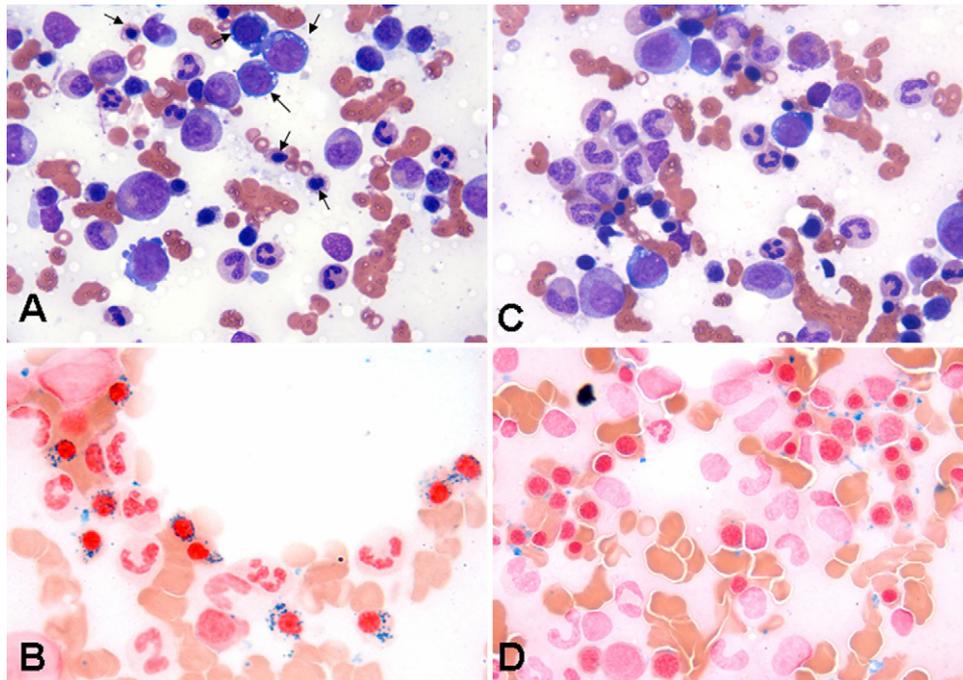


Fig. 1. Bone marrow (BM) aspirate smears of patient #2. Initial BM smear (A) showed dyserythropoiesis with 70% ring sideroblasts (B) (arrows pointing to dysplastic erythroblasts). Follow up BM smear 20 days later after stopping α -interferon showed resolved dyserythropoiesis (C) and no significant number of ring sideroblasts (D).

which showed a 50–60% cellularity with trilineage hematopoiesis and 1% RS (Fig. 1C and D). Two months after the first BM examination, his CBC nearly recovered with Hb 11.4 g/dL, MCV 86 fL; WBC $11.2 \times 10^9/L$, ANC $10.9 \times 10^9/L$ and platelets $163 \times 10^9/L$. Due to progression of MM, the patient succumbed to death 16 months later, without sign of BM failure.

3.4. Patient #3

An 82-year-old man with a past medical history significant for prostate cancer, status post surgery and radiation 11 years was newly diagnosed with angioimmunoblastic T-cell lymphoma (AITL). He was at least stage III by radiographic studies and underwent BM biopsy for a full staging. His CBC at the time of BM biopsy showed WBC $6.3 \times 10^9/L$ with ANC $3.9 \times 10^9/L$, Hb 12 g/dL, MCV 101 fL, RDW 14.6, and platelets $179 \times 10^9/L$. His BM showed a 50% cellularity with no evidence of lymphoma; no dysplasia in megakaryocytes or granulocytes, but mild dyserythropoiesis with 20% RS. The karyotype was normal. Although a diagnosis of RARS, likely therapy related because of prior radiation history, the patient showed no significant cytopenia. He received cyclophosphamide, hydroxydaunorubicin hydrochloride, vincristine and prednisone (CHOP) for AITL and achieved complete remission. However, he soon experienced lymphoma recurrence, and underwent BM restaging. At the time of restaging, he had WBC $3.6 \times 10^9/L$ with ANC $3.1 \times 10^9/L$; Hb 13.3 g/dL with MCV 97 fL and platelets $133 \times 10^9/L$. His BM showed no evidence of lymphoma, a 30% cellularity with trilineage hematopoiesis, <5% RS and no evidence of dysplasia. Flow cytometry immunophenotyping specifically designed to assess MDS CD34 cells was normal. Conventional karyotyping was normal; and FISH was negative for 20q or 13q abnormalities. Mutation studies for *RAS*, *FLT3*, *NPM1*, and *CEBPA* were negative. He returned to be cared by local oncologist, and lost follow-up.

3.5. Patient #4

This was a 67-year-old man with a history of stage IV follicular lymphoma, status post 6 cycles of fludarabine, mitoxantrone, dexamethasone and rituximab (FND-R) followed by maintenance α -interferon. He continuously had waxing and waning lymphadenopathy, and later showed disease progression and was diagnosed with B-lymphoblastic lymphoma/leukemia. He received induction chemotherapy with hyper-CVAD. He achieved complete remission, and received two courses of consolidation chemotherapy, and transitioned to maintenance phase chemotherapy with POMP regimen (6-mercaptopurine, vincristine, methotrexate and prednisone) concurrently with intrathecal methotrexate for CNS prophylaxis. He underwent BM reassessment after completion of 16 cycles of maintenance therapy. His Hb was 10.4 g/dL, MCV 96 fL, RDW 21.7%, WBC $3.9 \times 10^9/L$ with ANC of $3 \times 10^9/L$ and platelet count $22 \times 10^9/L$. BM examination revealed a 50% cellularity, 1% blasts, relative erythroid hyperplasia, mild dyserythropoiesis with 55% RS, but no significant dysplasia in other lineages. Karyotyping was normal. He soon was diagnosed with diffuse large B cell lymphoma in the liver. Another BM biopsy was performed for staging 4 weeks following the previous BM biopsy. His BM showed trilineage hematopoiesis, no dysplasia and <1% RS. There was no evidence of leukemia or lymphoma. He received single standard dose of rituximab infusion with good response and proceeded with monthly chemoimmunotherapy with vincristine and rituximab up to the last follow-up. He had had 5 BM biopsies for lymphoma and leukemia follow-up afterwards. All were negative for dysplasia or RS. The patient had been alive to the last follow-up (three years after the RARS-diagnosis) with latest Hb of 15.5 g/dL.

3.6. Patient #5

This 70-year-old woman was diagnosed with Philadelphia positive B-lymphoblastic leukemia (B-ALL) and received induction chemotherapy with hyper-CVAD and Dasatinib concurrently with intrathecal chemotherapy with methotrexate and cytarabine. She achieved complete remission after induction, and proceeded to consolidation therapy with the same regime. Her treatment course was complicated by neutropenic fever, pneumonia and urinary tract infection treated with vancomycin. A BM was performed per the protocol requirement for assessment of tolerance and further planning. At the time of BM biopsy, her CBC showed Hb 10.4 g/dL, MCV 87 fL, RDW 14.9, WBC $3.1 \times 10^9/L$ with ANC $2 \times 10^9/L$ and platelet count $231 \times 10^9/L$. BM examination revealed a 40% cellularity, 1% blasts, no megakaryocytic or granulocytic dysplasia, but mild dyserythropoiesis with 60% RS. Karyotype was normal. She was diagnosed with t-MDS (RARS), but required no management for cytopenia. She continued with consolidation therapy for B-ALL. A BM biopsy was performed 60 days later which showed mild dyserythropoiesis with no RS. The patient completed consolidation, maintenance therapy and continued with reduced dose of Dasatinib for B-ALL. She had another 14 BM biopsies performed over the following three years that all were negative for B-ALL or dysplasia. Her CBC was normal at the last follow-up.

3.7. Patient #6

The patient was a 54-year-old man with a past medical history of squamous cell carcinoma (SCC) of the tongue, treated with radiation and Cetuximab (anti-EGFR) 5 years ago. He was recently diagnosed with hepatitis C and cirrhosis secondary to blood transfusion due to a motor vehicle accident 10 years ago. He was treated with Ribavirin and weekly pegylated α -interferon and developed cytopenia. He received Procrit, and later red blood cell transfusion for symptomatic anemia. At the time of BM biopsy, his CBC showed WBC $1.7 \times 10^9/L$ with ANC $1.1 \times 10^9/L$, Hgb 6.8 g/dL, MCV 106 fL, and platelet $143 \times 10^9/L$. His BM showed a 20% cellularity, mild dysmegakaryopoiesis and mild dyserythropoiesis with 15–20% RS. Chromosomal analysis was normal. Although a pathology diagnosis of t-MDS was rendered, the management decision was made to discontinue Ribavirin and pegylated interferon and support with red cell transfusion. One week after discontinuation of Ribavirin and interferon, his Hb increased to 11 g/dL, and he became red cell transfusion independent. Six weeks after cessation of hepatitis C treatment, his CBC completely recovered and sustained. However, he was diagnosed with hepatocellular carcinoma 8 months later, and died within a year of that diagnosis. No follow-up BM biopsy was ever indicated.

4. Comparison between t-MDS and cases with transient/reversible RS

The laboratory data, BM findings, and clinical features of the 6 patients with transient/reversible RS were compared with 10 patients with true t-MDS with RS (Table 1). There was no difference in hemoglobin (Hb), absolute neutrophil count (ANC) or platelet count. Mean corpuscular volume (MCV) was comparable; however, red cell distribution width (RDW) appeared to be borderline lower in the transient/reversible RS patients ($p=0.07$). Storage iron was increased in both groups. The BM cellularity and BM blast percentage were not significantly different between these two groups, whereas erythroid hyperplasia appeared to be more frequently observed in t-MDS cases; although this difference was not statistically significant, perhaps due to small sample size ($p=0.11$). Significantly, dysplasia involving non-erythroid lineages

was more frequently observed in t-MDS than in cases with transient/reversible RS ($p=0.04$).

The median interval from initiating cytotoxic therapy to the diagnosis of t-MDS was 63 months (range, 14–251 months); whereas in patients with transient/reversible RS, the median time interval was 33 months (range, 6–132 months) ($p=0.369$). However, the latter group received multiple various treatments either for disease recurrence/progression or other medical conditions. With a median follow-up of 20 months (range, 0–95 months), 6 of 10 patients with t-MDS died, including 2 patients who died of progression of t-MDS and 3 patients who died from their original malignant neoplasms. The demise of one patient was only known by the report of death without comment on cause of death. One patient received allogeneic stem cell transplant and was alive at the last follow-up. In the transient/reversible RS group, 3 patients died, including 2 who died of progression of their primary cancer (patients #1 and #2) and 1 patient (patient #6) who died of another unrelated malignancy. None of the patients in the transient/reversible RS group died of BM causes.

5. Discussion

In this study, our goal was to assess the clinical significance of RS in the BM in the absence of other evidence of dysplasia in the post-therapy setting. We therefore focused specifically on a subgroup of patients who had significant (at least 15%) RS in the BM without elevated blasts (<5%) and who had a normal karyotype. In a retrospective review of 843 patients diagnosed with t-MN or suspicious of t-MN, we identified 19 (2.3%) patients who met the selection criteria. A total of 13 patients truly had MDS, therapy related in 10 patients and probably coincidental, not related to therapy in 3 patients. The remaining 6 patients had recovery of their peripheral blood counts and the follow up BM examination showed resolution of RS and associated dyserythropoiesis, indicative of a non-hematopoietic stem neoplasm but transient/reversible changes. We therefore conclude that the presence of significant number of RS, in the absence of increased BM blasts or cytogenetic abnormalities must be interpreted with caution.

Over interpretation of RS as evidence of MDS in the setting of cancer treatment may attributable to several factors. In some patients, a detailed clinical history and treatment information are not available at the time of BM assessment. The time sequence of onset of cytopenia in relation to the initiation of cytotoxic therapy may not be clear. Three cases in our series were likely to be coincident primary MDS but diagnosed as t-MDS due to insufficient clinical information at the time of diagnosis. Furthermore, in the 2008 WHO classification, although it is suggested that t-MN should not be diagnosed within 6 months of cytotoxic therapy, in reality many cancer patients receive multiple/various chemotherapies either for disease recurrence/progression; or undergo long-term consolidation or maintenance therapies. t-MN do arise during the course of these therapies, and a diagnosis of t-MN is not uncommonly made within 6 months of cessation of therapy. It is noteworthy that many neoadjuvant agents have been incorporated in cancer therapeutic regimens, and their effect on hematopoiesis is largely unknown. The complexity of cancer treatment can make establishing a diagnosis of t-MDS challenging, and this particularly true in patients with a normal karyotype and no increase in BM blasts. Importantly, although transient/reversible RS can be secondary to a number of drugs, the association of RS as a direct result of chemotherapy has been only rarely reported [9,10]. Instead, the presence of RS often implicates abnormal iron metabolism, a common feature of MDS, indicating of t-MDS in this setting [13–15].

Table 2
Known agents causing transient/reversible ring sideroblasts.

Causative agents	References
Alcohol	Sheftel et al. [24]; Latvala et al. [25]; Budde et al. [26]; Vetsch et al. [27]
Benzene	Sheftel et al. [24]; Netelson [28]
Lead poisoning	Sheftel et al. [24]; Conso et al. [29]
Copper deficiency and zinc overdose (leading to copper deficiency)	Gregg et al. [30]; Gill et al. [31]; Hayton et al. [32]; Simon et al. [33]; Fiske et al. [34]; Broun et al. [35]
Anti-tuberculosis drugs and other antibiotics (isoniazid; pyrazinamide; chloramphenicol; cycloserine; D-penicillamine, fusidic acid, tetracycline, linezolid, lincomycin)	Sheftel et al. [24]; Sharp et al. [36]; Vetsch et al. [27]; Vial et al. [5]; Rudek et al. [7]; Abena et al. [37]
Busulfan	Fernandez et al. [9]; Magalhaes et al. [10]
Pregnancy/progesterone	Natelson [3]; Bencaiova et al. [4]

Are there any features helpful in distinguishing cases with transient/reversible RS from true t-MDS with RS? The median time interval from initiation of cytotoxic therapy in the transient/reversible RS group was not significantly different from the true t-MDS group ($p=0.389$). The demographic information, BM cellularity, BM blast count, and cytopenia(s), either by severity or involved lineages, were similar between these two groups. However, we did find that RDW in the transient/reversible RS cases was not as high as the true t-MDS cases. This is in keeping with the theory that since the presence of RS was transient/reversible, it might have not affected the heme synthesis significantly to produce many dimorphic red cells as seen in sideroblastic anemia. In addition, we also observed some morphologic differences. True t-MDS cases more frequently showed dysplasia involving the non-erythroid lineages and therefore are more akin to refractory cytopenia with multilineage dysplasia (RCMD) rather than RARS. In contrast, in the transient/reversible RS group, only 1 patient (case 6) showed mild dysmegakaryopoiesis in addition to dyserythropoiesis and RS. In the follow-up BM specimens, whereas RS disappeared in the transient/reversible RS group, RS and dysplasia persisted in patients who had unequivocal t-MDS.

Normally, iron is stored as H ferritin in siderosomes in erythroblasts that are called sideroblasts [16,17]. The number of these H ferritin-type sideroblasts typically increases whenever the iron supply to erythroblasts exceeds the amount required for hemoglobin synthesis. Compared with physiological sideroblasts, the iron granules in RS represent mitochondrial ferritin (MtF). Cazzola et al. demonstrated a perinuclear distribution of MtF in patients with X-linked sideroblastic anemia and MDS with RS, but not in healthy controls or MDS without RS [18,19]. However, it has been known that RS can be observed in a number of conditions (Table 2) other than MDS and hereditary sideroblastic anemia; and the pathophysiology in each condition may differ [3]. In hereditary sideroblastic anemia, RS formation is largely attributed to missense mutations in *ALAS2* gene leading to defect protoporphyrin IX synthesis [20,27]. Drugs and toxins, such as ethanol, isoniazid, pyrazinamide and cycloserine are antagonists to pyridoxine, a coenzyme of aminolevulinic acid synthase (ALAS) in heme synthesis [3]. Copper enhances iron absorption in the gastrointestinal tract, cellular iron uptake from transferrin and iron incorporation into protoporphyrin IX [24], and copper deficiency [7–11] can be associated with RS. In MDS patients with RS, a primary defect in mitochondrial iron metabolism has been proposed, and acquired mutations in subunits of cytochrome oxidase encoded by mitochondrial DNA have been detected in some patients [24]. Gene expression data have shown that CD34+ cells from patients with RARS are characterized by upregulation of mitochondrial-related

genes and, in particular, those involved in heme synthesis (e.g. ALAS2) [21]. We have previously shown that del(11)q is associated with iron overload and RS in MDS patients, and loss of function of *ATM* and ferritin-related genes located in chromosome 11 long arm may be contributing factors [22]. As we believe that the 6 patients in the study do not have true t-MDS, it seems likely that drug therapy is a potential explanation for the transient/reversible appearance of RS in these patients. The patients in the study group received a broad spectrum of therapeutic agents before onset of RS, including various chemotherapeutic agents, antiviral Ribavirin, α -interferon, Vancomycin, Lenalidomide, Dasatinib, and Paclitaxel. Although these agents have not been previously reported to cause RS, we suspect that many of the newer therapeutic agents simply may have not yet made to the list of known causes of RS. Interestingly, 4 of 6 patients received α -interferon therapy either for primary malignancies (patients #1, #2, #4) or hepatitis C (patient #6). α -Interferon can cause cytopenia(s), however, no link with RS has been reported to date [23].

In summary, the presence of RS in the absence of increased BM blasts or karyotypic abnormalities is uncommon in the post-therapy setting, occurring in approximately 2% of all cases. Although most patients with significant number of RS did have MDS, in approximately one third of these patients, RS were transient/reversible and the patients did not show evidence of t-MDS on follow up. The presence of transient/reversible RS in these patients is currently unexplained, but drugs, particularly α -interferon were suspects. We conclude that RS in the absence of increased BM blasts or cytogenetic abnormalities must be interpreted cautiously, particularly in the absence of dysplastic changes in leukocytes or megakaryocytes. In this circumstance, careful chart review and recommendation for follow-up BM aspiration and biopsy is appropriate.

Conflict of interest statement

All authors have no conflict of interest to declare.

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