Case Report

A Rare Case of Myelodysplastic Syndrome with Ring Sideroblasts, SF3B1 and TET2 Mutations in a Patient with Beta Thalassemia Trait

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Concurrent myelodysplastic syndrome (MDS) and β-thalassemia trait is rare. We reported a case of a 59-year-old man with a known history of β-thalassemia (presumed to be β-thalassemia intermedia) presenting with progressive anemia and worsening fatigue. β-globin mutation analysis revealed a heterozygous mutation in the beta-globin gene, compatible with β-thalassemia trait. Bone marrow biopsy showed erythroid hyperplasia and erythroid dysplasia with increased ring sideroblasts (>15%). Additionally, there is also mild myeloid and megakaryocytic dysplasia. Molecular analysis revealed SF3B1 and TET2 mutations. These findings are consistent with myelodysplastic syndrome with ring sideroblasts (MDS-RS). To the best of our knowledge, this is the first report of MDS-RS with SF3B1 and TET2 mutations in a patient heterozygous for β-globin gene mutation. For β-thalassemia patients with worsening anemia, a comprehensive bone marrow analysis including cytogenetic and molecular studies is important to help further delineate the diagnosis.

INTRODUCTION

β-thalassemia and Myelodysplastic syndrome with ring sideroblasts (MDS-RS) are two distinct clinical entities that may present in a similar clinical and laboratory picture. β-thalassemia is a group of disorders characterized by mutations of the β-globin gene leading to reduced hemoglobin synthesis.[1] In the heterozygous state, or β-thalassemia trait, the clinical symptoms can vary from asymptomatic to mild to moderate microcytic anemia. MDS-RS is an acquired clonal defect in hematopoiesis characterized by anemia, erythroid dysplasia, and ≥5% ring sideroblasts with SF3B1 mutation or ≥15% ring sideroblasts without SF3B1 mutation.[2] There are rare case reports of concurrent Myelodysplastic syndrome (MDS) and β-thalassemia trait.[3-5] We report here a case of development of MDS-RS with SF3B1 and TET2 mutations in a patient with heterozygous β-globin mutation.

Case Report

A 59-year-old man, former long distance runner, with a reported history of β-thalassemia intermedia of an unknown genotype was referred to be evaluated for a splenectomy in the setting of progressive anemia, worsening fatigue, weight loss of 10 pounds over one year, and right upper quadrant pain.

Complete blood count showed hemoglobin 7.0 g/dL, hematocrit 22.2%, MCV 70.1 fL, red blood cell distribution width (RDW) 35.3%, WBC 5.9×10^3/ul (neutrophils 53%, lymphocytes 34%, monocytes 13%, nucleated RBC 1/100), platelets 287×10^3/ul, and reticulocytes 1.3%. Review of the peripheral blood smear showed anisopoikilocytosis, hypochromic RBCs, codocytes (target cells), dacrocytes, microspherocytes, basophilic stippling, pseudo Pelger-Huet cells, and giant platelet (**Figures 1A and 1B**). Iron studies revealed increased serum iron (209 ug/dl), increased serum ferritin (1443 ng/ml), and decreased total iron binding capacity (212 ug/dl). Serum lactic dehydrogenase (LDH) was within normal range. Decreased haptoglobin of <8 mg/dL and a blood smear revealing spherocytes indicated active hemolysis. Gallstones were found on ultrasound. Patient reported never having received a blood transfusion. Hemoglobin electrophoresis demonstrated Hemoglobin A of 94.2%, a mildly elevated Hemoglobin A2 of 4.0%, and Hemoglobin F of 1.8%. A laparoscopic cholecystectomy was performed for symptomatic cholelithiasis without complication. Two units of packed red blood cells were given prior to the procedure for hemoglobin of 7.6 g/dL with response to 9.4 g/dL.

Alpha and beta globin gene mutations were performed revealing heterozygosity for GLN39X beta-globin mutation and no alpha-globin mutation, compatible with Beta thalassemia minor. Patient was placed on Hydroxyurea with no improvement seen over the course of two months. Bone

marrow biopsy was then performed showing hypercellular marrow (90-95%) with absolute erythroid hyperplasia **(Figures 1C and 1D)**, erythroid dysplasia and greater than 15% ring sideroblasts **(Figure 1)**. There is no increase in blasts. There is marked dyserythropoiesis including megaloblastoid changes, nuclear-cytoplasmic asynchrony, irregular nuclear contour, nuclear binucleation, nuclear bridging, and nuclear budding **(Figures 1F and 1G)**. A few small hypolobated megakaryocytes are also seen but represent less than 10% of all megakaryocytes. There is mild myeloid dysplasia including hypogranulation and few pseudo pelger-Huet cells. Karyotype analysis showed one abnormal metaphase (53, XY, +4, +5, +9, +10, +18, +21) among 20 normal metaphases. Fluorescence *in situ* hybridization (FISH) for MDS panel was normal. Myeloid molecular

profile by targeted sequencing (Genoptix, CA) revealed pathogenic SF3B1 and TET2 mutations. Taken together, this is consistent with MDS-RS. Patient was classified as low-risk MDS clinically according to the International Prognostic Scoring System (IPSS). Patient continued to have stable but symptomatic anemia and was pursuing eligibility in a clinical trial for Luspatercept. Prior to clinical trial enrollment, a second bone marrow biopsy was performed. This showed similar findings with a slight increase of dysplastic megakaryocytes including small hypolobated and micromegakaryocytes **(Figures 1E and 1H; 10-20% of all megakaryocytes)**. Karyotype analysis of the second marrow biopsy was normal. Patient has been put on the clinical trial and is clinically stable now.
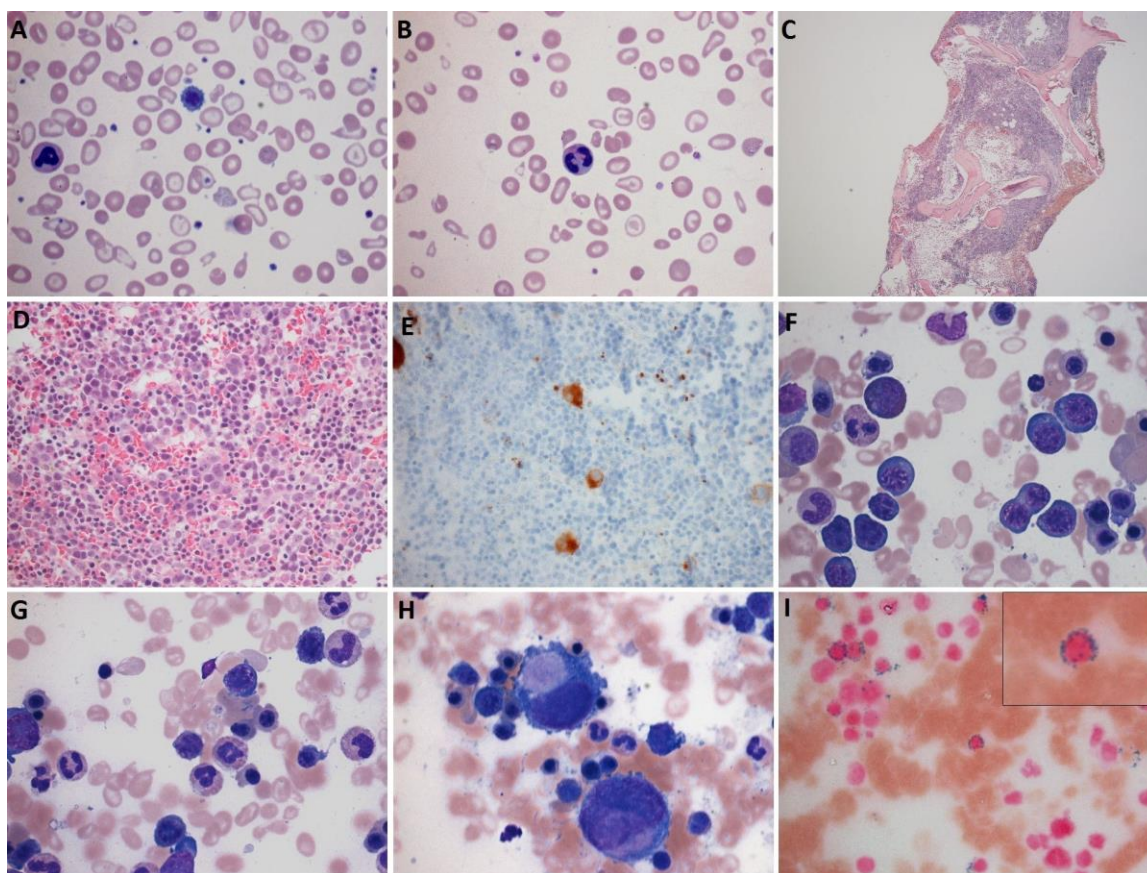

Figure 1. Images of peripheral blood smear and bone marrow biopsy/aspirate. **A & B.** Peripheral blood smear showing anisopoikilocytosis, codocytes (target cells), microspherocytes, basophilic stippling, pseudo Pelger-Huet cells, and giant platelet. **C.** Bone marrow core biopsy with hypercellular marrow. **D.** Bone marrow core biopsy with erythroid hyperplasia. **E.** Immunohistochemistry of core biopsy with CD61 demonstrating small hypolobated and micromegakaryocytes. **F-H.** Bone marrow aspirate with erythroid hyperplasia, erythroid precursors with megaloblastoid changes, irregular nuclear contour, binucleation, nuclear bridging, and nuclear budding; pseudo Pelger-Huet cells, and small hypolobated megakaryocytes. **I.** Bone marrow aspirate with Prussian blue iron stain demonstrating ring sideroblasts. (original magnifications, x1000 [A-B, F-I], x40 [C], x400 [D-E])

DISCUSSION

Concurrent β-thalassemia and myelodysplastic syndrome is rare. Niscola et al. reported 9 cases of MDS in alpha- or beta-thalassemia patients; none of these 9 patients had increased ring sideroblasts.[4] There was a case report of ring sideroblasts

found in a bone marrow biopsy of a 5-year-old girl with β-thalassemia trait.[5] However, percentage of ring sideroblasts was not specified in the case report and the etiology of ring sideroblasts was not clear.[5] There was another case report of

refractory anemia with ring sideroblasts and thrombocytosis (RARS-T, WHO 2008; Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis, revised WHO classification, 2016) in a patient with beta thalassemia intermedia.[3] However, concurrent beta-thalassemia trait and definite diagnosis of MDS-RS has never been reported.

Both β-thalassemia and MDS-RS can present as anemia and show hypercellular marrow with erythroid hyperplasia in the bone marrow biopsy. β-thalassemia presents as microcytic anemia[1] while MDS-RS usually presents as normocytic or macrocytic anemia.[6] MDS-RS usually has morphologic dysplasia in erythroid lineage and may be accompanied by myeloid and megakaryocytic dysplasia in some cases. However, it can be challenging to differentiate MDS from reactive etiologies of dysplasia.[7,8] Although the threshold to define dysplasia is 10% dysplastic cells in any hematopoietic lineage, it has been shown that 10% or more dysplastic cells can be seen in reactive conditions.[9] Thus cytogenetic and molecular studies are important to help define the diagnosis. In this case, the first bone marrow karyotype analysis showed one abnormal metaphase among 20 metaphases while the second karyotype analysis was normal. The findings of only one abnormal cell in single culture indicate that this is probably an artifact. Molecular study by targeted sequencing showed SF3B1 mutation and TET2 mutation. Taken together, this is consistent with the diagnosis of MDS-RS.

Frequent SF3B1 (splicing factor 3b subunit 1) mutations are detected in MDS and are particularly associated with MDS-RS.[10,11] MDS with SF3B1 mutation generally carries good prognosis whether it is single lineage or multilineage dysplasia in the absence of excess blasts.[12] Malcovani et al. showed no significant difference of clinical phenotype and outcome in patients with less than 15% ring sideroblasts compared to patients with great than 15% ring sideroblasts.[12] In the revised WHO classification, only 5% ring sideroblasts are needed for MDS-RS if SF3B1 mutation is detected, while 15% or more ring sideroblasts are still required in cases without SF3B1 mutation.[2]

Recent studies have shed light on the mechanism how SF3B1 mutations affect the formation of ring sideroblasts in MDS.[13-15] The SF3B1 protein is a major component of the U2 small nuclear ribonucleoproteins (snRNPs), which is involved in the recognition of the branch site (upstream of 3' splice site) during pre-mRNA splicing. Mutations in SF3B1 could result in the misrecognition of 3' splice site, and subsequent aberrant mRNA splicing.[13] This further leads to either degradation of downstream targets through nonsense-mediated RNA decay (NMD) pathway or accumulation of aberrant proteins. The expression of many genes was affected by SF3B1 mutation in MDS-RS. Notably, few mitochondria-related genes were significantly affected.[14,15] One of the mitochondria-related genes, the iron transporter ABCB7, showed marked downregulation in MDS-RS, which may result in the abnormal iron accumulation seen in ring sideroblasts.[15]

It was also found that mutations in DNA methylators are associated with multilineage dysplasia.[12] TET2 (ten-eleven translocation-2) plays an important role in DNA methylation and is a critical regulator in hematopoiesis.[16] TET2 mutations are frequently found in variety of myeloid neoplasms, including MDS, acute myeloid leukemia, and chronic myelomonocytic leukemia (CMML).[17] Until recently, the mechanism how TET2 mutation affects DNA methylation and contributes to tumorigenesis was not clear. By using the mouse model and *in vitro* cell culture studies, Rasmussen et al. showed that TET2 mutation leads to hypermethylation of active enhancers throughout the genome, which in turn regulates the expression of tumor suppressor genes and contribute to leukemogenesis.[17] In MDS, TET2 mutations are not particularly associated with cytopenia or blast count and are generally associated with favorable or neutral prognosis.[18,19] In our case, there is multilineage dysplasia with the TET2 mutation but no neutropenia and thrombocytopenia clinically. Patient has low-risk MDS according to IPSS. The molecular profile is also associated with good prognosis.

In summary, to the best of our knowledge, this is the first case report of mixed MDS-RS with SF3B1 and TET2 mutations and β-thalassemia trait. Both β-thalassemia trait and MDS-RS can present with a similar clinical and laboratory picture. For patients with progressive anemia and other symptoms, it is important to have comprehensive bone marrow analysis including cytogenetic and molecular studies to obtain a definitive diagnosis.

CONFLICT OF INTEREST
The authors have no conflicts of interest to disclose.

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