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Case Report

Refractory Anemia with Ring Sideroblasts Associated with Marked Thrombocytosis Complicated by a Parasternal Granulocytic Sarcoma

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Abstract

Refractory anemia with ring sideroblasts associated with marked thrombocytosis (RARS-T) and granulocytic sarcoma are two rare myeloid neoplasms. RARS-T is a Myelodysplastic/Myeloproliferative Neoplasm (MDS/MPN) characterized by overlapping myeloproliferative (platelets count higher than $450x10^9$/L associated with proliferation of large atypical megakaryocytes similar than those observed in essential thrombocythemia) and myelodysplastic features (dysplastic erythroid proliferation with ring sideroblasts >15%). RARS-T molecular pattern also exhibited myelodysplastic and myeloproliferative overlap with frequent *SF3B1* and *JAK2* mutations, justifying its classification in MDS/MPN disorders. Granulocytic Sarcoma also referred as myeloid sarcoma or chloroma are rare extramedullary tumors of immature myeloid cells that partially or totally efface the tissue architecture. Extramedullary involvements include a large variety of clinical significant phenomena that often pose diagnosis problems. Granulocytic sarcoma may develop *de novo* or concurrently with acute myeloid leukemia or myeloproliferative neoplasms. However, no previous case of granulocytic sarcoma associated to myelodysplastic/myeloproliferative neoplasms has been reported. Herein, we report a case of RARS-T revealed by an asymptomatic thrombocytosis complicated by a granulocytic sarcoma ten months after initial diagnosis. This extramedullary megakaryoblastic mass invaded the sternum and the parietal soft tissues without associated myeloid leukemia.

ABBREVIATIONS

MDN: Myelodysplastic Neoplasm; MPN: Myeloproliferative Neoplasms; AML: Acute Myeloblastic Leukemia; RARS-T: Refractory Anemia with Ring Sideroblasts and Marked Thrombocytosis; GS: Granulocytic Sarcoma; ET: Essential Thrombocytosis; SF3B1: Splicing Factor 3B Subunit1

INTRODUCTION

Myelodysplastic/Myeloproliferative Neoplasms (MDS/MPN) are rare clonal myeloid proliferations that show overlapping myeloproliferative and myelodysplastic features at initial presentation [1]. Refractory Anemia with Ring Sideroblasts associated with Thrombocytosis (RARS-T) is included in MDS/MPN and retained as a provisional entity of the World Health Organization classification of myeloid neoplasms (WHO 2008) [2,3]. Diagnostic criteria for RARS-T are ineffective erythropoiesis with more than 15% of ring sideroblasts and prominent myeloproliferative features with a marked thrombocytosis (higher than $450x10^9$/L) associated with the proliferation of large atypical megakaryocytes in the bone marrow. Moreover, a careful exclusion of well-defined Myelodysplastic (MDN) and Myeloproliferative Neoplasms (MPN) is essential before establishing the diagnosis of RARS-T. In particular, an exhaustive molecular and cytogenetic work-up should exclude the presence of t (9;22) or *BCR-ABL* transcript identifying a chronic myeloid leukemia, Acute Myeloid Leukemia (AML) defining translocations

[e.g., inv(16)(p13.1q22)/t(16;16)(p13.1;q22), t(8;21)(q22;q22) or inv3(q21;q26.2)/t(3;3)(q21;q26.2)], and isolated del(5q) that is more supportive of a diagnosis of MDS [4]. RARS-T cases frequently harbor *SF3B1* (Splicing factor 3b, subunit 1) mutations that are implicated in ring sideroblasts formation [5]. Indeed, *SF3B1* mutations are found in about 20% of total MDS whereas they are strongly associated with the presence of ring sideroblasts with a frequency of 64-82% in RARS and 66-72% in RARS-T. *SF3B1* and *JAK2* mutations are the main prognostic factors in RARS-T and are associated with a better survival [6]. Here, we report a case of *JAK2*-negative RARS-T complicated with a Granulocytic Sarcoma (GS) of megakaryoblasts involving the sternum and the parietal soft tissues. GS are rare extra-medullary tumors of immature myeloid cells that partially or totally efface the tissue architecture [7,8]. Although bone, skin, or lymph nodes are the most frequent localizations, any part of the body may be affected [9]. Those extra-medullary involvements often pose a diagnostic dilemma [10]. GS may develop *de novo*, concurrently with AML or more rarely with MPN [11]. However, no previous case of GS associated with RARS-T has been reported.

CASE PRESENTATION

A 57-year-old woman was referred for the exploration of an asymptomatic thrombocytosis. In January, her complete blood count showed are generative macrocytic anemia and confirmed thrombocytosis (Hemoglobin 10g/dL, mean corpuscular volume 100fL, reticulocytes 75x10^9/L, Platelets 1,500x10^9/L, leucocytes 6.5x10^9/L). The bone marrow aspirate smears showed a markedly increased number of large megakaryocytes, frequently occurring in cluster, associated with large platelet clumps. They exhibited deeply lobulated nuclei and abundant mature cytoplasm. Emperipolesis of marrow elements in the cytoplasm of megakaryocyte was observed on the smears (Figure 1A). Otherwise, these myeloproliferative features were concomitant with a marked dysplasia in the erythroid lineage. Erythroblasts exhibited a defective hemoglobinization with laminated cytoplasm, and nuclear abnormalities (hypercondensation, nuclear lobulation). In addition, 68% of ringsideroblasts were visualized with Perls stain (Figure 1A). Finally, blasts represented fewer than 5% of nucleated cells and did not exhibit morphologic abnormalities. Moreover, the bone marrow trephine biopsy was hypercellular and revealed a proliferation of large to giant megakaryocytes occurring in loose cluster without blast excess. The network of reticulin fibers was increased, revealing a diffuse grade 2-3 myelofibrosis (Figure 1B). *BCR-ABL* transcript, *JAK2-V617F* and *CALR* (Calreticulin) mutations were negative. The karyotype was normal. We identified a *SF3B1* mutation (c.1997A>G: p.K666R). In conclusion, the presence of such hybrid myeloproliferative and myelodysplastic features with more than 15% of ring sideroblasts met criteria for *JAK2*-negative RARS-T. A treatment with pipobroman (Vercyte®) was initiated and quickly replaced by anagrelide (Xagrid®) due to poor clinical tolerance (erythema).

In November, the patient presented with sternal pain associated with rapid loss of weight and anemia (Hb 8g/dL; platelets 150x10^9/L and leucocytes 20x10^9/L without circulating blast). Computed tomography identified an osteolytic bone lesion of the inferior third of the sternum and a mass invading

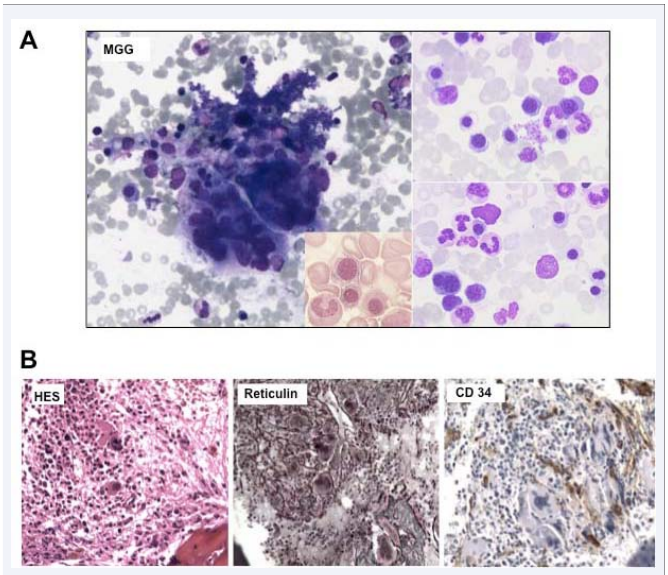

Figure 1 Bone marrow aspirate and biopsy showing Refractory Anemia with ringsideroblasts and marked thrombocytosis (RARS-T)

A: Bone marrow aspirate smears reveal an increase in the number and size of megakaryocytes with deeply lobulated (stag-horn-like) nuclei and large platelets clumps. Note that pictures on the right pictures show an important dyserythropoiesis with multinuclearity, cytoplasm vacuolization and ring sideroblasts after Perls stain.

B: Bone marrow trephine biopsy confirms the proliferation of giant megakaryocytes showing arrangement in clusters. Those cells can be stretched by the underlying diffuse fibrosis (silver stain). CD34 staining shows enlarged and stretched vessels and confirms the absence of blast excess (<5%).

pectoral muscle and local soft tissues. A surgical biopsy of this mass revealed a severe fibrotic process that massively invaded the striated muscle and a proliferation of atypical cells (Figure 2). These cells were of medium to large size, with a unique large round nucleus, rarely bi-nucleated with nucleoli and a clear chromatin. Some cells were larger and exhibited megakaryoblastic differentiation. This tumoral infiltrate occurred in large clusters separated by fibrotic patches. An exhaustive immunohistochemistry study showed a high CD34-positivity and reacted with antibodies to megakaryocyte-restricted antigens CD41, CD31 and Factor VIII. All the cells strongly expressed CD41 and CD31, whereas factor VIII expression was restricted to the larger atypical megacaryocytes (Figure 2). The pan-leukocyte marker CD45, myeloid (MPO, CD15, CD117) and lymphoid (CD20, CD79a, CD3, CD5) antigens were absent. These features matched with a megakaryoblastic granulocytic sarcoma. The concomitant bone marrow aspirate did not show evidence of blast excess.

Induction chemotherapy with idarubicin and aracytine resulted in a rapid decrease of the sternal mass on CT scan. However, nine months later, the patient presented with a mandibular osteolytic mass and femoral neck fracture treated by surgical osteosynthesis with hip screw. Histological analysis of the surgical reaming products revealed an infiltration by megakaryoblasts, exhibiting the same phenotype as previous sternal tumor. This relapse of granulocytic sarcoma was treated

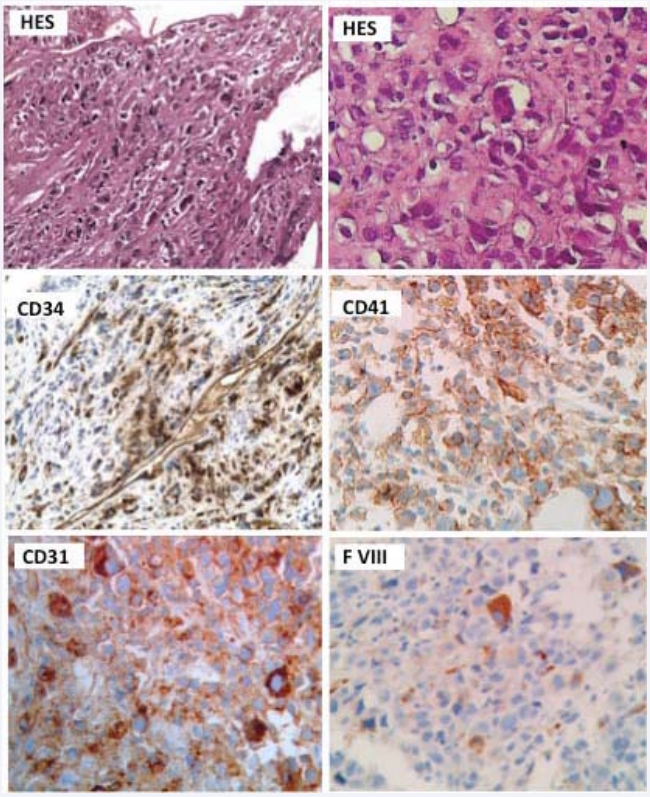

Figure 2 Sternal mass biopsy revealing a megakaryoblastic granulocytic sarcoma.
Medium to large sized cells with unique large round nuclei, rarely bi-nucleated, presenting with a clear chromatin and occurring in clusters separated by large fibrotic patches. These cells reacted with antibodies to CD34 and the megakaryocytes-related antigens CD41, CD31 and Factor VIII (for the most differentiated cells).

with EMA (etoposide, mitoxantrone and aracytine) with a poor response and fatal outcome.

DISCUSSION

RARS-T and SG are two rare clonal myeloid proliferations currently defined in the WHO 2008 classification [2]. Herein, we report a unique case of RARS-T complicated by a megakaryoblastic GS.

Before the characterization of RARS-T in the WHO 2008 classification, this entity was recognized as an unclassifiable MDS/MPN, also called Essential Thrombocythemia with Ring Sideroblasts (ET-RS) in the WHO 2001 classification. It was known to have an intermediate survival between essential thrombocythemia and the other unclassifiable MDS/MPN [12]. Before the discovery of the involvement of *JAK2* mutations, the prognostic evaluation of RARS-T was only guided by the morphological evaluation of bone marrow. Thus, "myeloproliferative-type" RARS-T (as described in our clinical cases) was regarded as having a better prognosis than "dysplastic-type" or "primary myelofibrosis" RARS-T [12]. Then, several studies showed that *JAK2-V617F* mutation together with a platelet count higher than $600x10^9$/L, were good prognostic factors [13-16].

Since 2008, RARS-T is morphologically defined by a combination of objective signs of erythroid dysplasia (ring sideroblasts) and megakaryocytes with proliferative pattern. On the molecular level, recent sequencing studies have identified the role of RNA-splicing machinery in the pathogenesis of MDS [17,18]. Thus, mutations in the *SF3B1* gene are involved in the formation of ring sideroblasts and are found in about 80% of cases of RARS-T [5,19,20]. Furthermore, the *JAK2-V617F* mutation is found in over 60% of RARS-T, regardless of *SF3B1* mutations [19,21]. The frequency of *CALR* mutations in RARS-T seems to be lower than in ET or primary myelofibrosis [17]. *TET2, ASXL1* and *EZH2* mutations show similar frequencies as those observed in MDS and a higher occurrence of *DNMT3A* has been reported in RARS-T [17,22]. *JAK2-V617F* and *SF3B1* are independent factors associated with better prognosis and a higher median survival whereas *ASXL1, DNMT3A* and *EZH2* mutations are associated with poorer outcomes [6,22]. Concordantly, our case of *JAK2*-negative RARS-T presented an unfavorable outcome with an early transformation in GS and therapeutic failure.

Finally, the prognosis of patients with RARS-T is more pejorative than ET, justifying a systematic Perls staining in the assessment of thrombocytosis, particularly if it is associated with erythroid dysplasia. Indeed, AML rarely complicates ET, particularly in patients younger than 60 years old [23]. In this report, the proliferation of dysplastic megakaryocytes on bone marrow biopsy, severe thrombocytosis (>$450x10^9$/L) fulfilled the diagnostic criteria of ET. However, the identification of >15% of ring sideroblasts, together with the *SF3B1* mutation provide evidence in the diagnosis of RARS-T.

RARS-T transformation in a granulocytic sarcoma is an exceptional condition. Megakaryoblastic GS can rarely complicate ET with frightening outcome [24]. However, to our knowledge, the association of RARS-T with GS has never been described. The diagnosis of SG is often difficult and requires an histological study with comprehensive immunochemistry [10]. In this case, CD34 and CD41 positivity enabled the diagnosis of megakaryoblastic GS without bone marrow involvement. CD34 and CD41 are found positive in 74% and 90% of megakaryoblastic leukemia respectively, but the lack of other myeloid markers is unusual [25]. CD31 and Factor VIII, also known as endothelial markers, can be used to detect normal and atypical megacaryocytes [26]. Megakaryoblastic GS is generally localized in bones and adjacent soft tissues [24,27]. Thus, GS has to be suspected when facing an isolated lytic bone lesion in the context of known MDS/MPN, even without biological signs of leukemic transformation. The diagnosis of GS requires intensive treatment whatever the bone marrow status because of their constant transformation in AML [28].

CONCLUSION

RARS-T is a very rare myelodysplastic/myeloproliferative disorder. Perls staining may be systematic for the assessment of thrombocytosis, especially in association with dyserythropoiesis, in order to exclude the diagnosis of RARS-T. Today, the prognosis of RARS-T depends on the presence of the *JAK2, SF3B1* and *ASXL1* mutations.

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