

REFRACTORY ANAEMIA WITH RINGED SIDEROBLASTS ASSOCIATED WITH MARKED THROMBOCYTOSIS (RARS-T) WITH SUPERIMPOSED 5Q-SYNDROME

MATEUSZ ZIARKIEWICZ¹, JADWIGA DWILEWICZ-TROJACZEK¹, ANNA PASTWIŃSKA², ELŻBIETA CHMARZYŃSKA², MAŁGORZATA PASZKOWSKA-KOWALEWSKA¹, ŁUKASZ KOPERSKI³, WIESŁAW WIKTOR JĘDRZEJCZAK¹, BOGNA ZIARKIEWICZ-WRÓBLEWSKA^{3,4}

¹Department of Haematology, Oncology and Internal Diseases, Warsaw Medical University

²Department of Paediatric Pathology, Cytogenetics Laboratory, Warsaw Medical University

³Department of Pathology, Warsaw Medical University

⁴Department of Pathology, Institute of Oncology in Warsaw

Refractory anaemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T) is a rare entity belonging to myeloproliferative/myelodysplastic syndromes. Myelodysplastic syndrome (MDS) with isolated del(5q) is a category of MDS characterized by better prognosis and specific morphology. Herein we describe a 69-year-old male with anaemia and thrombocytosis presenting with coexisting features of both these rare diseases. After the description of the clinical data, we summarize the histopathologic, cytogenetic and molecular findings, as well as introduced treatment. Next, we discuss possible diagnostic options with reference to the relevant literature.

Key words: RARS-T, 5q- syndrome, MDS with isolated del(5q).

Introduction

Refractory anaemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T) is a provisional entity described in the third edition of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, which constitutes a subgroup belonging to a broader category of unclassified disorders with overlapping features of myelodysplasia and myeloproliferation (MDS/MPN, U). This syndrome is characterized by myelodysplastic changes in bone marrow morphology with concomitant increase in megakaryocytes and thrombocytosis $\geq 450 \times 10^9/l$, presence of ringed sideroblasts ($> 15\%$ of the erythroid precursors) and less than 5% of blasts in the bone marrow and $< 1\%$ in the peripheral blood [1-4].

Myelodysplastic syndrome with isolated del(5q) is a subtype of MDS with the deletion of the long arm of chromosome 5 without any other cytogenetic abnormalities, which typically presents with

anaemia with or without other cytopenias and thrombocytosis. This entity has relatively good prognosis and low incidence of transformation to acute myeloid leukaemia. Transfusion independence is frequently achieved on lenalidomide treatment [1, 5, 6].

Herein we present a case with coexistence of these two separate entities.

Case presentation

Clinical data

A 69-year-old man with a history of hypertension and diverticulosis presented to his family doctor with general weakness lasting for several months. On physical examination pallor of the skin and mucosa was noted, as well as splenomegaly. His CBC revealed microcytic anaemia (Hgb 9.3 g/dl, Ht 30%, RBC $4.2 \times 10^{12}/l$, MCV 67 fL, MCH 32 pg/dl, MCHC 33 g/dl), leukocytosis (WBC $19.1 \times 10^9/l$) and thrombocytosis (platelet count $650 \times 10^9/l$). Peripheral

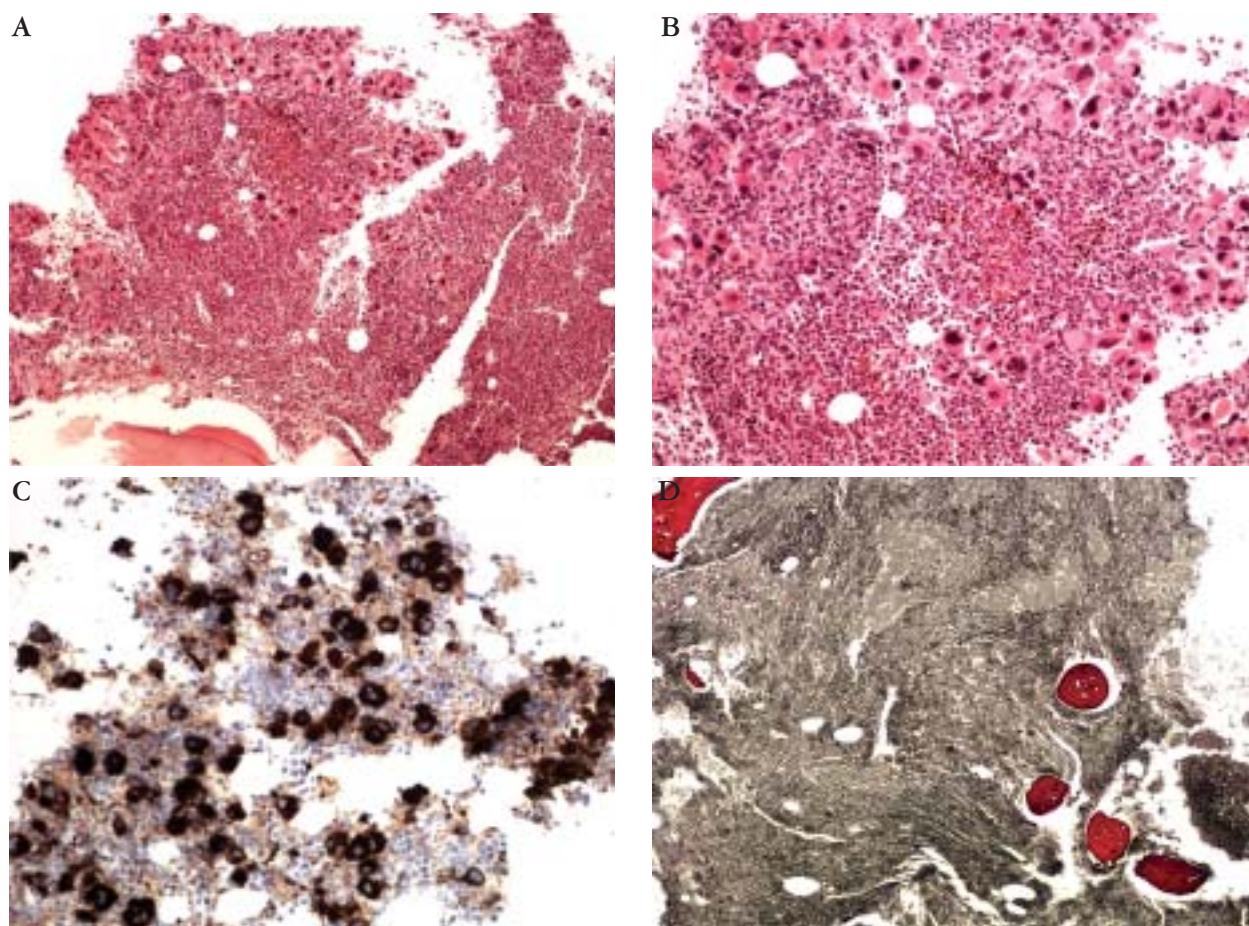


Fig. 1. Initial bone marrow trephine biopsy. A – hypercellular bone marrow with an increased number of megakaryocytes. B – morphology of megakaryocytes is consistent with 5q- syndrome. C – megakaryocytes show strong expression of CD 61. D – gomori stain revealed fibrosis of the bone marrow

blood smear showed a left shift in neutrophils (promyelocytes 1%, myelocytes 1%, metamyelocytes 2%, bands 2%, neutrophils 68%, lymphocytes 26%). Iron and ferritin concentrations were slightly diminished (Fe 60 $\mu\text{g}/\text{dl}$, ferritin 12 $\mu\text{g}/\text{l}$).

Bone marrow morphology and immunophenotype

Initial bone marrow cytological examination disclosed hypercellularity with 15.6% of erythropoietic cells, 77.6% of granulopoietic cells, 3.2% of monocytes, 4.0% of lymphocytes, 1.2% of blasts and numerous megakaryocytes. Ringed sideroblasts constituted 35% of erythroid precursors. Trephine biopsy (Fig. 1) showed hypercellular bone marrow (haematopoietic to fatty tissue: 90 : 10) with fibrosis (2/3 in 4 grade scale). Erythropoiesis to myelopoiesis ratio was 1 : 6. Erythropoiesis was reduced, though normoblastic, without evident dyserythropoiesis. Granulopoiesis was excessive with normal maturation. CD34+ cells and CD117+ cells constituted less than 5% of all cells. The most prominent feature was numerous atypical megakaryocytes of different

size and shape, many with hypolobated nuclei, which were abnormally located and formed loose and dense clusters (Fig. 1B, C). Many of them displayed typical morphology for MDS with isolated del(5q), namely non-lobated or hypolobated, rounded nuclei. They showed expression of megakaryocytic markers, like FVIII or CD61 (Fig. 1C). Additionally, foci of necrotic tissue were noted.

During the following 2 years, three consecutive trephine biopsies were performed. In the second biopsy half a year later, bone marrow was still hypercellular, though to a lesser extent.

Megakaryocytes were still prominent, though at this time they acquired expression of CD34. Moreover, immature myeloid precursor (CD 34+, CD 117+) appeared to be increased. Erythropoiesis showed some dysplastic features (Fig. 2). Fibrosis did not change substantially. Sideroblasts were even more prevalent (49%). Immunophenotyping of bone marrow cells demonstrated 2% of cells in the region of blasts (CD45/SSC). Blast cells expressed myeloid markers, though expression of HLA-DR was unusually low. Next two biopsies, 5 and 10 months later,

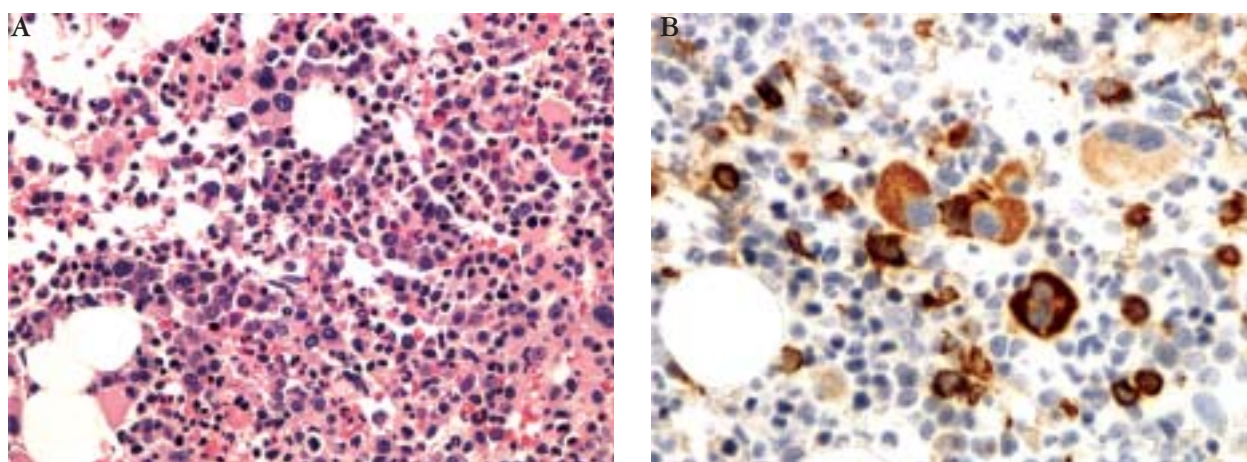


Fig. 2. Second bone marrow trephine biopsy. A – features of mild dyserythropoiesis. B – megakaryocytes are strongly CD34-positive

disclosed a sustained decrease in fibrosis (stage 2 in 4 grade scale). Atypical megakaryocytes CD34+ still predominated. Dyserythropoiesis was observed and so was an increased percentage of immature myeloid precursors. Immunophenotype analysis disclosed 4.5% of cells in the blast region (CD45/SSC).

Molecular and cytogenetic findings

Cytogenetic analysis of the material taken at the initial presentation revealed the deletion of the long arm of the 5th chromosome (del 5q31), without any other associated abnormalities. Del 5q31 was detected in all 30 analyzed metaphases. These abnormality was confirmed subsequently 3 times in cytogenetics and using FISH technique with specific probe EGR1(5q31).

Apart from that, further genetic investigations were undertaken for differential diagnosis of myeloproliferative and myelodysplastic syndromes. Translocations BCR-ABL p210 t(9;22) (q34;q11), marker of chronic myeloid leukaemia (CML), and ETV6-PDGFRB t(5;12) (q33;13), characteristic of myeloproliferative syndromes with eosinophilia, were excluded by RT-PCR method. JAK2 V617F mutation, typically present in polycythaemia vera (PV), as well as in 50% of cases of essential thrombocytopenia (ET) and primary myelofibrosis (PMF), was not detected using RQ-PCR method (real-time quantitative PCR). Additionally, FISH analysis did not reveal any p53 gene deletions.

A cell culture *in vitro* from bone marrow aspirate did not show signs of growth either spontaneously in methylcellulose semi-solid medium or after the addition of G-CSF and GM-CSF.

Diagnosis and treatment

On the basis of the collected data, a diagnosis of RARS-T with superimposed 5q- syndrome was pro-

posed. First line treatment was hydroxyurea during 2 months. After diagnosis of 5q- syndrome, lenalidomide was introduced. At present the patient is treated with lenalidomide 10 mg in cycles lasting 21 days with a 7-day break and hydroxyurea 500-1000 mg daily.

After 1.5 year it seems that partial response has been achieved, which manifests in improvement of blood parameters (independence of transfusions) and bone marrow morphology. Most notably, a significant decrease in the fibrosis stage was observed. However, no cytogenetic response was detected.

Discussion

The presented case showed overlapping features of a myelodysplastic syndrome and a myeloproliferative neoplasm. In differential diagnosis several conditions should be considered, which include myelodysplastic/myeloproliferative neoplasm, unclassifiable (MDS/MPN, U), RARS-T, PMF and MDS with isolated del 5q and their combinations [7].

Thrombocytosis associated with hypercellular bone marrow with an increased percentage of megakaryocytes and significant fibrosis is suggestive of primary myelofibrosis. Described bone marrow morphology may be interpreted as an early fibrotic stage of the disease. However, morphology of megakaryocytes in this case was not consistent with PMF, where they are characterized by large size with an abnormal pattern of chromatin clumping and “cloud-like” nuclei and usually form dense clusters adjacent to the bone trabeculae and vascular sinuses [1, 8]. Absence of the JAK2 V617F mutation and relative decrease in fibrosis, as well as presence of sideroblasts and evolving dysplastic features further argue against this diagnosis [9].

On the other hand, presence of the 5q deletion as a single cytogenetic abnormality, abundant small

megakaryocytes with conspicuously hypoblated nuclei and < 5% myeloblasts in the bone marrow warrant a diagnosis of MDS with isolated del(5q) [1, 5, 6]. Moreover, thrombocytosis and anaemia are also described in this disease. Minor severity of dysplasia in the initial biopsy may also weigh in favour of the 5q-syndrome, as myeloid and erythroid lineages in this entity are rarely affected [5]. However, bone marrow fibrosis together with an increasing percentage of immature myeloid precursors and dyserythropoiesis in subsequent biopsies as well as presence of sideroblasts suggest that single diagnosis of 5q-syndrome in this case would be inappropriate.

An interesting feature of the presented case is the presence of ringed sideroblasts. Their percentage increased from 30% at presentation to 49% and 70% later on. An increased percentage of ringed sideroblasts, megakaryocytic hyperplasia, anaemia and thrombocytosis are features of a provisional entity separated from the subgroup of myeloproliferative/ myelodysplastic syndromes – RARS-T [1-4]. In order to establish this diagnosis, sideroblasts should be detected from the beginning, so that it is clear that they are not a result of therapy or disease progression [10]. Up to 60% of cases with this syndrome have the JAK2 V617F point mutation [3]. However, it has been found negative in this patient. Cases with deletion of 5q are excluded from this category according to the WHO criteria, thus it cannot fully describe the presented case.

Herein we propose a diagnosis of RARS-T with superimposed 5q- syndrome. Concomitant presence of these two entities would most completely describe the presented case, encompassing both the myelodysplastic and myeloproliferative component. Fibrosis has been previously described in patients with RARS-T [9]. It should be regarded as an unspecific feature, commonly associated with megakaryocytic proliferation.

Szpurka *et al.* [4] described a retrospective series of patients with RARS-T. In one patient with normal morphology of megakaryocytes and no signs of fibrosis, del(5q) was found. However, authors were the opinion that the presence of JAK2 V617F mutation and thrombocytosis in that case supported the diagnosis of RARS-T.

It should be noted that an interesting feature in this case was the intense expression of CD34 on megakaryocytes (see Figure 2B), which was absent in the first biopsy, but was detected in subsequent biopsies with an upward tendency. Normally, CD34 is expressed only on immature precursors of megakaryocytes [11]. Some authors suggest that increased expression of CD34+ on megakaryocytes should be regarded as a feature of MDS, especially if it is found in >20% of these cells [12]. However, others argue that this phenomenon of aberrant phe-

notypic maturation is unspecific, appearing also in other myeloid neoplasms or even reactive conditions [11]. In our opinion, it should be regarded as an interesting feature of myelodysplasia, seen also in megakaryoblastic leukaemia, though it must be interpreted with caution.

Lenalidomide, an immunomodulatory agent, is a 4-amino-glutamyl analogue of thalidomide that lacks its neurologic side effects and has emerged as a drug with activity against multiple myeloma and MDS with isolated del(5q) [13]. In the latter disease, it may reduce transfusion requirements and induce complete cytogenetic response in up to 60% of cases. Moreover, there are also reports describing its successful use in RARS-T [14]. Its efficacy was partially confirmed in the described case. Although cytogenetic response was not achieved, on-treatment biopsies showed a sustained decrease in bone marrow fibrosis with improvement of peripheral blood morphology. Antifibrotic effect of lenalidomide has been already described in a small cohort of patients with PMF [15].

This case was presented during the 9th International Course on Bone Marrow Pathology in Geneva in 2009. No clear consensus regarding the diagnosis was achieved in the course of the panel discussion. We describe it herein to show that despite the diversity of haematopoietic neoplasms presented in the latest WHO Classification, diseases which do not fit well to any category still exist. Treatment of these patients remains a big challenge for clinicians.

References

1. World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th edition, IARC Lyon 2008.
2. Gupta R, Soupir CP, Johari V et al. Thrombocytosis with sideroblastic Erythropoiesis: a mixed myeloproliferative myelodysplastic syndrome. *Leuk Lymphoma* 34: 615-619.
3. Hellstrom-Lindberg E, Cazzola M. The Role of JAK2 Mutations in RARS and Other MDS. *Hematology Am Soc Hematol Educ Program*. 2008; 52-59.
4. Szpurka H, Tiu R, Murugesan G, Aboudola S, et al. Refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T), another myeloproliferative condition characterized by JAK2 V617F mutation. *Blood* 2006; 108: 2173-2181.
5. Washington LT, Doherty D, Glassman A, et al. Myeloid disorders with deletion of 5q as the sole karyotypic abnormality: the clinical and pathologic spectrum. *Leuk Lymphoma*. 2002; 43: 761-765.
6. Giagounidis AA, Germing U, Haase S, et al. Clinical, morphological, cytogenetic, and prognostic features of patients with myelodysplastic syndromes and del(5q) including band q31. *Leukemia* 2004;18: 113-119.
7. Thiele J, Kvasnicka HM. Clinicopathological criteria for differential diagnosis of thrombocythemias in various myeloproliferative disorders. *Semin Thromb Hemost*. 2006; 32: 219-230.
8. Thiele J, Kvasnicka HM. Hematopathologic findings in chronic idiopathic myelofibrosis. *Semin Oncol* 2005; 32: 380-394.

9. Tefferi A, Lasho TL, Schwager SM, et al. The JAK2 (V617F) tyrosine kinase mutation in myelofibrosis with myeloid metaplasia: lineage specificity and clinical correlates. *Br J Haematol* 2005; 131: 320-328.
10. Sheftel AD, Richardson DR, Prchal J, Ponka P. Mitochondrial iron metabolism and sideroblastic anemia. *Acta Haematol* 2009; 122: 120-133.
11. Orazi A. Histopathology in the diagnosis and classification of acute myeloid leukaemia, myelodysplastic syndromes, and myeloblastic/myeloproliferative diseases. *Pathobiology* 2007; 74: 97-114.
12. Pellegrini W, Facchetti F, Marocolo D, et al. Expression of CD34 by megakaryocytes in myelodysplastic syndromes. *Haematologica* 2000; 85: 1117-1118.
13. List A, Dewald G, Bennett J, et al. Lenalidomide in the Myelodysplastic Syndrome with Chromosome 5q Deletion. *N Engl J Med* 2006; 355: 1456-1465.
14. Huls G, Mulder AB, Rosati S, et al. Efficacy of single-agent lenalidomide in patients with JAK2(V617F) mutated refractory anemia with ring sideroblasts and thrombocytosis. *Blood*. 2010 Mar 1. [Epub ahead of print]
15. Quintás-Cardama A, Kantarjian HM, Manshour T, et al. Lenalidomide plus prednisone results in durable clinical, histopathologic, and molecular responses in patients with myelofibrosis. *J Clin Oncol* 2009; 27: 4760-4766.

Address for correspondence

Mateusz Ziarkiewicz
Department of Haematology, Oncology and Internal Diseases
Warsaw Medical University
ul. Banacha 1a
02-090 Warszawa

THE AUTHORS OF CORRECT DIAGNOSIS ARE:

Paulina Dominik MSc, Department of Pathomorphology, Regional Hospital in Jelenia Góra,
ul. Ogińskiego 6, 58-506 Jelenia Góra

Małgorzata Trzcńska MD, Provincional Hospital No2 in Rzeszów, Department of Pathomorphology,
ul. Lwowska 60, 35-301 Rzeszów

Kamil Winnik MD, Janusz Korczak Memorial Provincial Specialist Hospital in Słupsk,
Department of Pathomorphology, ul. Hubalczyków 1, 76-200 Słupsk