

Peculiarities in the Diagnosis Approach of MDS /MPN-U Patients

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ABSTRACT

The most recent WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues describes a set of diseases framed as the MDS / MPN (myelodysplastic / chronic myeloproliferative syndromes). There are four subtypes comprised in this category: chronic myelomonocytic leukemia, juvenile myelomonocytic leukemia, atypical chronic myeloid leukemia and unclassifiable MDS / MPN. They combine both myelodysplastic and myeloproliferative features. Although the unclassifiable MDS/ MPN subtype specifically associates the myelodysplastic and myeloproliferative features, it does not meet the criteria defining the first 3 subtypes. The RARS-T subtype (refractory anemia with ringed sideroblasts associated with marked thrombocytosis) is included in the MDS / MPN-U as a temporary entity. There are two cases described in this article: one diagnosed with RARS-T and one with MDS / MPN-U. Both cases evolved towards acute myeloid leukemia.

INTRODUCTION

The unclassifiable MDS/ MPN diagnosis is valid for a patient that initially associates both myelodysplastic and myeloproliferative alterations. While the myelodysplasia is characterised by ineffective hematopoiesis with peripheral cytopenia, the myeloproliferative process is associated with an overproduction of mature cells. The genetic alterations seems to activate the conversion to acute leukemia. □

CASE REPORT I

The patient is a 66 year-old man, admitted in our clinic on the 17th of November 2008 with: headache, fatigue, dizziness. His medical history was notable for an anemic syndrome first diagnosed in 2001 as a medium macrocytic anemia. Paraclinical assessment showed normal serum dosage of vitamin B12 and folate, normal serum iron level, normal upper and lower gastrointestinal endoscopy. His lifestyle revealed a balanced diet. He had never

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been chronically exposed to cigarettes, alcohol and/ or any toxic treatment. At the admittance in our department, the clinical examination showed good general physical condition and mucocutaneous pallor. **Blood tests:** medium macrocytic anemia, leucopenia and thrombocytosis (Hb = 8.7 g/dl, MCV = 110 fl, WBC = 3800/mm³, PLT = 429000/mm³), peripheral blood smears with fragmented erythrocytes, macrocytic RBCs; without blasts cells; pseudo Pelger- Huet abnormality; negative Coombs tests and absent antieritrocytes antibodies; serum iron and ferritin values were increased. **Bone Marrow Smear Test** hipercellularity with dysplastic changes of erythroid and myeloid lineage (Figure 3,4); 2% blasts cells, megakaryocytic hiperplasia (Figure 1); *Prussian blue staining* - ringed sideroblasts 41% (the exceeding values of more than 15% ringed sideroblasts define the refractory anemia with ringed sideroblasts- a subtype of myelodysplastic syndrome) (Figure 2). **Bone marrow biopsy** Histological aspect - hipercellularity, M / E = 5/1; **megaloblastoid erythropoiesis**, the left shift deviation, bilobed granulocyte nuclei- pseudo Pelger- Huet abnormality, without dysplastic changes of megakaryocytic lineage. Immunohistochemical tests- do not show increased percentage of CD 34 positive cells, but positive myeloperoxidase staining. Cytogenetic examination - structural and numerical complex karyotype abnormalities-hypodiploidy -45 chromosomes in 65%: 45 (monosomy: -8[2]; -17[7]; -21[2]; -y[2]; trisomy: +8[2]; +20[3]; deletions: del(4)(q31)[3]); without presence of del 5q witch could explained thrombocytosis. **Diagnosis** - Myelodysplastic syndrome- refractory anemia with ringed sideroblasts. **Differential diagnosis** - ringed sideroblasts associated disorders- alcoholism, antituberculostatic therapy, arsenic and zinc poisoning, pyridoxine or copper deficiency, mitochondrial disorders. **Treatment management:** erythropoietin therapy and hematological clinical monitoring.

Medical assessment. The increased anemic syndrome required for red blood packed cells transfusion. Other laboratory parameters that had a significant impact on patient's clinical status were: secondary serum iron and ferritin increased values (due to transfusion requirements), hepatocytolysis, mildly elevated blood sugar level. Abdominal MRI revealed iron liver deposits and hepatosplenomegaly. Secondary diagnosis - Posttransfusional haemo-

siderosis - required for specific therapeutical conduct - oral iron chelator, in order to help prevent comorbidities and improve life expectancy. Medical reevaluation two years after the primary diagnosis: Bone marrow smear: multilineal dysplasia with high percentage of marrow ringed sideroblasts (50%). Bone marrow biopsy- histological and immunohistochemical characteristics- adequate age cellularity, the M: E ~ 3:1 ratio, present but reduced maturing features, diserythropoiesis, abnormal localization of immature precursors, low (2% cells) CD 34 positive, megakaryocytic hiperplasia. Given the persistent thrombocytosis (654000/mm³), a molecular test was recommended. The test revealed the JAK2 mutation.

Diagnosis - Ringed sideroblasts refractory anemia associated with thrombocytosis

According to the latest WHO classification (2008), ringed sideroblasts refractory anemia associated with thrombocytosis (RARS-T), is a boundary entity. RARS-T is defined by the following criteria:

1. Refractory anemia associated with erythroid dysplasia and ringed sideroblasts 15% or greater;
2. Less than 5% blasts in the BM;
3. Platelet count $450 \times 10^9 / L$ or greater;
4. Presence of large atypical megakaryocytes similar to those observed in BCR/ABL1-negative MPN;
5. Absence of del (5q), t (3; 3) (Q21, Q26), or inv (3) (q21q26).

Medical assessment – one year - of RARS-T - CBC reveals pancytopenia with circulating blasts discharge (32%). BM smear shows blast cell infiltration -52%. Final diagnosis is acute myeloblastic leukemia postmyelodysplastic syndrome. The patient receives chemotherapy treatment without any therapeutic response. □

CASE REPORT II

The patient is a 65 year-old man, living in rural areas, without exposure to toxic factors, without any significant pathological history. **Clinical assessment** showed areas of skin necrosis at the toe and second finger from the left plant, pain and swelling. He presented himself to the territorial surgery department. The CBC performed highlighted important thrombocytosis (2500000/mm³). He was guided to the clinical hematology department. **The physical exam** detected tissue necrosis areas in

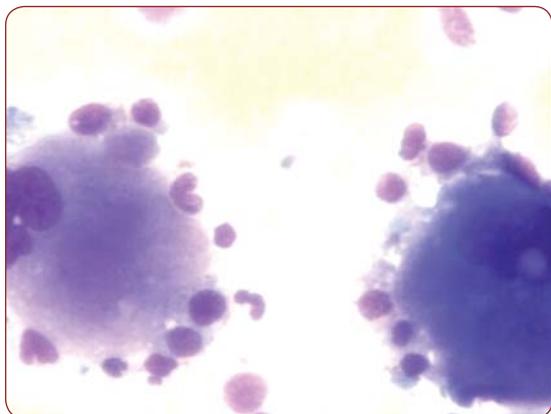


FIGURE 1. BM smear – May Grünwald Giemsa staining-frequent megakaryocytes

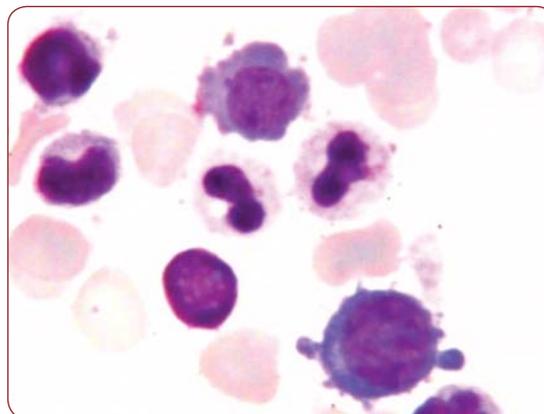


FIGURE 3. BM smear – May Grünwald Giemsa staining - erythrocyte and granulocytic dysplasia

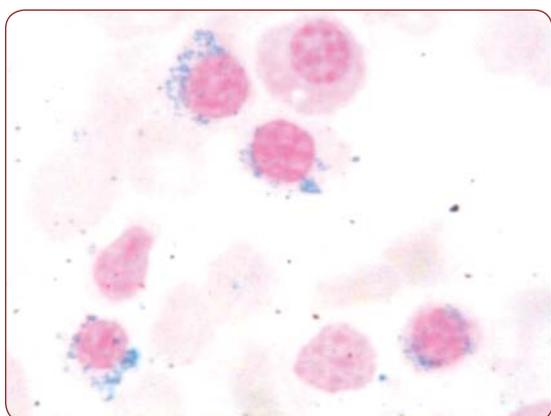


FIGURE 2. Perls staining showing numerous ring sideroblasts on BM smear. Magnification x 100. (Ringed sideroblasts are erythroblasts - their mitochondrias contain ferritin granules with a perinuclear array on more than one third of its circumference)

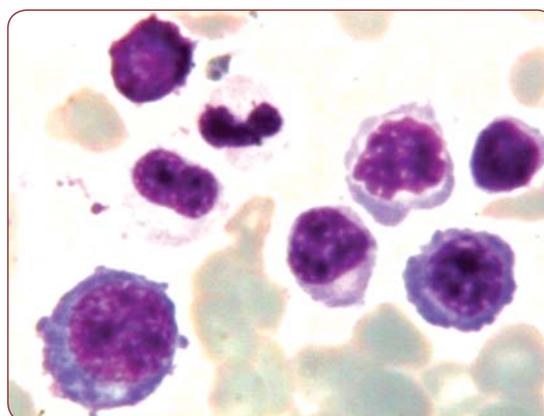


FIGURE 4. BM smear – May Grünwald Giemsa staining - erythrocyte and granulocytic dysplasia

the left big toe and second finger of the plant. It also revealed the splenic lower pole situated 3 cm below the costal rebord. **Laboratory tests:** CBC revealed considerably increased value of platelets (2000000/mmc) without anemia or leukopenia, normal leukocyte formula, LDH increased level. Abdominal ultrasound showed a splenic diameter of 15 cm. JAK-2 mutation detection was positive. **Bone marrow biopsy** confirmed the presence of a chronic myeloproliferative / myelodysplastic syndrome with a unilineal dysplasia of the erythroid lineage and megakaryocytic hyperplasia. Immunohistochemical tests- do not show CD 34 positive cells. According to these investigations the patient was diagnosed with chronic myeloproliferative / myelodysplastic syndrome- unclassifiable.

Differential diagnosis should include reactive thrombocytosis, other entities belonging to

the chronic myeloproliferative syndrome like: polycythemia vera, myelofibrosis with myeloid metaplasia, chronic granulocytic leukemia, essential thrombocythemia; different types of myelodysplastic syndrome (refractory anemia, ringed sideroblasts refractory anemia, refractory anemia with excess of blasts), chronic myelomonocytic leukemia, aplastic anemia.

The patient's clinical evolution was favorable under cytoreductive treatment.

Medical assessment. The patient returns after aprox. 1 year with physical fatigue and intense mucocutaneous pallor. **Biological status:** Hb-6, 2g/dl; WBC-15500/mmc, circulating blasts- 24%; PLT-81000/mmc, biological inflammatory syndrome and LDH increased values.

Bone marrow biopsy - histological and immunohistochemical aspect shows the left shift deviation, abnormal pattern of immature precursors (ALIP +), moderate diserythropoiesis, CD 34 positive cells ~20%, positive myeloperoxidase staining, rare functional megakaryocytes.

The diagnosis was myelodysplastic / chronic

myeloproliferative syndrome - unclassifiable, that turned to acute myeloblastic leukemia. We initiated adjuvant chemotherapy, packed red blood cells and erythropoietin transfusions. After 9 months, patients' condition associated posttransfusional hemochromatosis and we initiated treatment with iron chelators to lower iron loading. The patient is currently being supervised and haematologically assessed in order to detect any abnormal karyotype. □

DISCUSSION

CD 34 positive cells alterations in the gene coding for heme synthesis (up-regulation of ALAS2) and down-regulation of ABCB7 (this gene synthesizes a protein that helps with the iron pass from mitochondrias to cell cytoplasm) appeared in both RARS-T and RARS. Changes related to the expression of other genes like PSIP1-LEDGF, CXCR4 and CDC2L5 were only found in RARS-T. The most significant aspect though, is that ~50% of patients who suffered from RARS-T have a characteristic MPN gene mutation. This gene encodes JAK tyrosine kinase 2. Fewer cases described another specific MPN MPL gene mutation. The MPL gene encodes the thrombopoietin receptor synthesis. These peculiarities lead to the idea that RARS-T is a myeloid neoplasm that displays myelodysplastic and myeloproliferative features at both molecular and clinical levels. In light of these findings, as in the clinical case presented above, we could state that RARS-T is a developing

condition from RARS, by acquiring somatic mutations like JAK 2 and possibly other unknown mutations (3). Other comparative studies have concluded that patients with RARS-T have a similar prognosis to those diagnosed with RARS. Nevertheless, their clinical evolution is less favorable compared to essential thrombocythemia patients (2). This fact enhances the importance of differential RARS-T/ ET diagnosis for patients with normal haemoglobin values. The possible favorable prognosis implies the JAK 2 mutation and RARS subtype. The unfavorable prognosis of the case presented above, lies in the complex abnormalities of the karyotype that involve increased acute leukemia transformation and hepatic posttransfusional hemosiderosis risks. Cytogenetics studies have revealed changes with prognostic value. The good cytogenetics is normal cariotip or with -5q, -Y, -20q as sole abnormalities. The poor prognostic is in the presence of complex (ie, ≥3 abnormalities) or chromosome 7 abnormalities; all other abnormalities confer an intermediate risk (4). According to this results our patient associated complex cytogenetic abnormalities with poor prognostic and high risk to progress to AML.

The particularity of the last case lies in the debut manner of a myelodysplastic / chronic myeloproliferative syndrome with clinical and biological essential thrombocythaemia features. The delicate issue of achieving a certain final diagnosis had been solved through repeated bone marrow biopsies.

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