Myeloproliferative Disorders

Occurrence of the *JAK2* V617F mutation in the WHO provisional entity: myelodysplastic/myeloproliferative disease, unclassifiable -refractory anemia with ringed sideroblasts associated with marked thrombocytosis

The *JAK2*/V617F mutation has been noted in essential thrombocytemia. We investigated 19 cases with refractory anemia with ringed sideroblasts (RARS), including three RARS with thrombocytosis (RARS-T). Only the RARS-T patients showed this mutation. More cases need to be analyzed to determine the prevalence of the *JAK2*/V617F mutation in RARS-T.

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Chronic myeloproliferative diseases (MPD) are clonal hematologic malignant disorders of myeloid lineages. The criteria for diagnosing these disorders have been revised by the WHO. Apart from the classic MPD, including polycythemia vera (PV), essential thrombocytemia (ET) and idiopathic myelofibrosis (IMF), the WHO classification includes other MPD. PV and ET are characterized by an increased production of platelets and red cells.[1] Activation of tyrosine kinase pathways are implicated in the pathogenesis of MPD.[2] Hematopoietic cells of ET present a number of aberrations such as the growth of endogenous erythroid colonies (EEC) and overexpressed *PRV-1* mRNA.[3] Recently, a single mutation of *JAK2*, a cytoplasmic tyrosine kinase, was detected in most patients with PV and in half the patients with ET. A single point mutation (Val617Phe) dysregulates the kinase activity of *JAK2*.[4,5] This dysregulation induces the abnormal hematopoiesis.[4] A similar cohort of MPD patients shared the *JAK2* mutation and EEC formation and *PRV-1* overexpression.[6] The *JAK2* mutation is rare in cancers and hematopoietic disorders apart from classic MPD, except in myelodysplastic syndromes (MDS) developing myelofibrosis which probably indicates the myeloproliferative nature in a subset of MDS patients.[7] The WHO classification establishes a new category, the myelodysplastic/myeloproliferative diseases (MDS/MPD). This category includes myeloid disorders that have both dysplastic and myeloproliferative features. Unclassifiable (MDS/MPD,U) are included in this new category. MDS/MPD,U-refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T) is incorporated in this category as a provisional entity. The clinical and morphological features consist of the myelodysplastic syndrome, refractory anemia with ringed sideroblasts (RARS) but with a marked thrombocytosis (>600×10⁹/L). The megakaryocytes are enlarged in size, as in PV/ET.[1] We obtained DNA from blood samples from three patients with RARS-T. These samples were analyzed using the allele-specific PCR methodology described by Baxter EJ *et al*.[5] DNA from the HEL cell line was used as the positive control. DNA samples from 16 patients with RARS and from 21 with ET were also studied. The patients gave permission for this study.

Table 1. Characteristics of patients.

Age/sex.	*Year*	*Clinic*	*Hb g/L*	*MCV fL*	*Leu ×10⁹/L*	*Plat ×10⁹/L*	*Bone marrow*	*Cyto*	*EEC*	*Epo U/L*	*JAK2*
70 F	1990		105	103	5	196	50% ringed sideroblasts	–	–	–	–
	1995	1995 Thrombosis 1996 Hemorrhage Platelets decreased with hydroxyurea 2001 Splenomegaly	95	91	7.4	621@	hypercellularity giant megakaryocytes 68% ringed sideroblasts	–	–	–	–
	2005		81	96	6	53	Dry tap, fibrosis, giant megakaryocytes	no mitosis			V617F
82 F	2003	Platelets decreased with anagralide	105	91	9.5	1260	Hypercellularity Giant megakaryocytes 20% ringed sideroblasts	46,XX	Neg*	29	
	2005										V617F
67 F	1996	Platelets decreased with ^{32}P	124	88	6.7	739	Hypercellularity Giant megakaryocytes 40% ringed sideroblasts	46,XX	–	–	
	2003		86	100	6.6	783	Hypercellularity Giant megakaryocytes 70% ringed sideroblasts	46,XX/ 46,xx,13q-	Neg#	20	
	2005										V617F
Controls Age:72±8 M: 8; F: 8		RARS	94±16	105±7	5.5±2	211±102 (69-410)					Wild type

*Year: year of diagnosis and follow-up bone marrow studies. M: male, F: female Leu: leukocytes, Plat: platelets. Cyto: cytogenetics, EEC: endogenous erythroid colony formation in vitro. *16 BFU-E/10⁵ mononuclear cells with Epo, 0 without Epo. #12 BFU-E/10⁵ mononuclear cells with Epo, 0 without Epo. @Platelets ranged from 605 to 1607×10⁹/L. V617K: presence of V617F mutation of JAK2. RARS: refractory anemia with ringed sideroblasts.*

In the three cases with RARS-T, the V617F mutation of the *JAK2* gene was detected, but none of the other cases with RARS showed the mutation. Interestingly, *in vitro* endogenous erythroid colony formation was negative in two of them. Bone marrow examinations showed hypercellularity with prominent megakaryocytic proliferation, enlarged in size. None of them showed the typical small-sized megakaryocytes of the 5q- syndrome. After a long follow-up (15 years) one case evolved to myelofibrosis (Table 1). In the ET group, 13 out of 21 cases showed the *JAK2* mutation.

The WHO classification of hematologic malignancies established RARS-T as a provisional category. The expert hematologists of the WHO classification agreed that this name should be applied till future studies indicate a more exact classification. The expert group concluded that it remained to be ascertained whether this entity is a distinct syndrome or the simultaneous occurrence of two disorders (RARS and ET).[1] Our data confirmed that a MPD was present in the three cases. It goes without saying that further data from other groups are necessary to confirm the prevalence of the *JAK2* mutation in RARS-T. However, it is clear that a number of patients show features similar to MPD disorders, such as the *JAK2* mutation and the evolution to myelofibrosis.[3,4,5,7]

On the other hand, these patients share characteristics of RARS, such as macrocytic anemia and an erythroid dysplasia similar to that of RARS.[1] Moreover, semisolid clonogenic assay of BFU-E showed not only a lack of endogenous erythroid colony formation, but also a decrease in the BFU-E progenitors.[8]

Recently, a review of a series of consecutive cases with RARS-T highlighted the diagnostic criteria for this entity.[9] This study excluded cases with reactive thrombocytosis or secondary causes for ringed sideroblasts and pointed out the presence of bone marrow megakaryocytes with giant forms.[9] Our three cases met these criteria. It should be noted that the survival of patients with RARS-T resembled the survival plot of RARS, not that of ET.[9]

Bearing these data in mind, RARS-T appears to be the coexistence of two disorders, with erythropoiesis showing the characteristics of RARS and megakaryocytes those of ET. However, a link must be found between these diseases given the difficulty in explaining the number of cases with RARS-T. While this manuscript was submitted several cases with RARS-T and the *JAK2* mutation were communicated to the 47th Annual Meeting of the American Society of Hematology.[10]

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