MPL W515 and JAK2 V617 Mutation Analysis in Patients With Refractory Anemia With Ringed Sideroblasts And An Elevated Platelet Count

Discovery of a constitutively activating point mutation of the Janus kinase 2 (JAK2) receptor-associated tyrosine kinase in patients with polycythemia vera (PV) and other BCR/ABL-negative myeloproliferative disorders[1] prompted many groups around the world to examine diverse subsets of patients with myeloid diseases for the prevalence of the JAK2 V617F mutation and its clinical and pathological associations.

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One of the rarest clinicopathological entities recently subjected to JAK2 mutation analysis has been refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T),[2,3] a somewhat nebulous clonal myeloid condition that was recently reviewed by Shaw.[4] RARS-T is a provisional entity included by the World Health Organization (WHO) in its 2001 revised classification of hematopoietic disorders within the ill-defined *myelodysplastic/myeloproliferative disease, unclassifiable* cluster (MDS/MPD, U; ICD-O code 9975/3).[5] The WHO definition of RARS-T applies to patients whose marrow exhibits morphological features consistent with the more common MDS subtype RARS (i.e., dysplasia that is most prominent in the erythroid lineage, with >=15% of erythroid precursors represented by ringed sideroblasts), yet associated with atypical megakaryocytic proliferation and an elevated platelet count, usually >600×10^9/L (similar to the definition of essential thrombocythemia).[5-7] Patients with *5q- syndrome* or abnormalities of chromosome 3q21q26 – other uncommon forms of MDS that can be associated with thrombocytosis – are excluded from the WHO definition of RARS-T, as are those with secondary causes of sideroblastosis. Shaw has proposed expanding the definition of RARS-T to include patients with a platelet count of >500×10^9/L, while specifically excluding patients with iron deficiency, postsplenectomy state, >3% marrow blasts, abnormal cytogenetics, circulating blasts, or unremarkable megakaryocyte morphology.[4] None of these proposed definitions are strictly evidence-based, and the molecular etiology for RARS-T is unknown.

Recently, our chronic myeloid disorders working group, in conjunction with D. Gary Gilliland's scientific group in Boston, described several novel activating somatic point mutations in a conserved residue of the thrombopoietin receptor (MPL W515 mutations) in association with clonal myeloid disorders.[8] Assays of samples from 1182 patients revealed that these MPL mutations are present only in cells from individuals with essential thrombocythemia or chronic idiopathic myelofibrosis (approximately 5% of patients with IMF and 1% of patients with ET), while MPL W515 mutations are absent in hematopoietic tissues from PV, chronic myeloid leukemia, the common forms of MDS, and from healthy individuals. Interestingly, MPL W515 mutations were seen in conjunction with JAK2 V617F in several cases; the mutation may commit transformed cells towards a thrombopoietic phenotype rather than a pure erythropoietic, polycythemic phenotype.[8]

In view of the association of MPL W515 mutations with thrombocytosis and their association with JAK2 V617F, we examined patients with >=15% sideroblasts and an elevated platelet count for these mutations (Table). After obtaining Institutional Review Board permission and ensuring compliance with governmental medical record privacy guidelines, we isolated bone marrow genomic DNA from patients with sideroblastic anemia associated with a supranormal platelet count, and examined these samples for the presence of MPL W515 or JAK2 V617F mutations. For both JAK2 and MPL, LightCycler-based (Idaho Technology, Salt Lake City, Utah) polymerase chain reaction assays were used to detect mutations, as previously described;[8,9] additionally, we performed direct fluorescent dye-chemistry sequencing of the relevant area of JAK2.[10]

Amongst more than 1500 patients with marrow-confirmed MDS diagnosed at Mayo Clinic over a 6-year period, 132 cases of sideroblastic anemia were identified; only 10 of these had a sustained platelet count above the normal reference range in our laboratory (i.e., >450×10^9/L), and these 10 patients were analyzed further. Only 3 patients with ringed sideroblasts had platelet counts persistently >600×10^9/L. Two of the patients with sideroblastic anemia and platelet counts >450 but <600×10^9/L had undergone splenectomy, and hyposplenism likely accounted for their mild thrombocytosis. The karyotype was normal in all cases, except for one male patient with 45, X, -Y.

Two patients had JAK2 V617F (moderate mutant allele burden) – 2 of the 3 patients with a platelet count of 600×10^9/L - and all 10 samples were negative for MPL W515 mutations. None of these cases were included in our previous initial series of MDS/atypical MPD and JAK2 V617F.(10) JAK2 V617F-positive cases had no features distinguishing them from mutation-negative cases other than a trend toward a higher platelet count, though numbers are too small to be definitive.

Morphological re-review of the bone marrow from the cases included in this series disclosed that all were consistent with the WHO definition of RARS-T, except for the milder elevation of platelet count (<600×10^9/L) in 7/10 cases. The two patients with hyposplenism likely would be excluded in a stricter definition of RARS-T such as that proposed by Shaw, as would the one patient who had marked dysplasia in the megakaryocyte lineage as well as some granulocyte dysplasia. (This individual might be classified as *RCMD-RS-T* – refractory cytopenia with multilineage dysplasia with ringed sideroblasts and thrombocytosis – if such an entity existed). Finally, a few patients had scattered dual-esterase staining cells in their marrow; previous series describing RARS-T patients have not routinely included dual esterase staining, so the significance of this MDS-associated finding is uncertain.

In conclusion, both the biology of RARS-T and its relationship to MDS, essential thrombocythemia, or other chronic myeloid neoplasias remain mysterious. The present results confirm the occasional finding of JAK2 V617F in RARS with thrombocytosis described by others (6 of 9 patients in the study by Szpurka and colleages, and 3 of 3 patients in the report of Remacha and colleagues).[2,3] Our findings also highlight the extreme rarity of RARS-T as defined by the WHO and by Shaw (3/>1500 MDS cases, and 3/132 RARS, if one holds to the strictest proposed standards), the diversity of disorders within *MDS/MPD,U*, the considerable challenges presented to morphologists by unclassifiable cases, and our rapidly evolving collective insights into the molecular pathology of clonal myeloid disorders.

Table 1. Patients with >=15% ringed sideroblasts and an elevated platelet count analyzed for MPL W515 and JAK2 V617F.

Patient #	Age	Sex	Platelet count ($x10^9$/L)	Ringed sideroblasts (%)	Hb (g/dL)	MCV (fL)	Overall marrow cellularity (%)	Multilineage dysplasia?	Hypo-splenism?	Karyotype	JAK2 V617F?	MPL W515 mutation?
1	65	F	456	70	8.8	104	70	N	N	NI	N	N
2	52	M	484	60	11.7	114	80	N	Y	NI	N	N
3	78	M	490	30	9.7	99	80	N	N	45,X,-Y	N	N
4	72	F	505	40	10.9	111	70	N	Y	NI	N	N
5	85	F	506	60	9.0	112	60	N	N	NI	N	N
6	71	F	515	20	7.7	89	90	N	N	NI	N	N
7	74	M	559	70	8.3	93	80	Y	N	NI	N	N
8	70	M	609	60	11.4	98	70	N	N	NI	Y	N
9	70	F	631	50	9.3	100	60	N	N	NI	N	N
10	67	F	819	70	8.9	95	80	N	N	NI	Y	N

All cases had <5% marrow blasts and <1% circulating undifferentiated blasts. Platelet count in this table is that obtained at the time-point of sample obtained for molecular analysis; platelet count fluctuation (above normal range) was also observed over time. For "multilineage dysplasia?", cases with only dual esterase staining or minor neutrophil segmentation abnormalities are not counted. Abbreviations: Hb = hemoglobin, MCV = mean cell volume, NI = normal, JAK2 = Janus kinase 2, MPL = myeloproliferative leukemia virus oncogene (thrombopoietin receptor).

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