JAK2^{V617F} mutation status identifies subtypes of refractory anemia with ringed sideroblasts associated with marked thrombocytosis

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ABSTRACT

Background
Refractory anemia with ringed sideroblasts and marked thrombocytosis (RARS-T) was recently shown to be a *JAK2*-V617F mutation-related disorder. To determine the frequency and the prognostic significance of this mutation, we retrospectively evaluated 23 patients with platelet counts more than 600×10^9/L, 15% ringed sideroblasts or more, and at least erythroid marrow dysplasia.

Design and Methods
An allele-specific polymerase chain reaction for *JAK2*-V617F was used to determine the allelic ratio of the mutated *JAK2* allele in DNA samples extracted from bone marrow biopsies. Hematologic and survival data of the *JAK2*-V617F positive *vs.* the *JAK2*-V617F negative patients were statistically analyzed. Allele-specific polymerase chain reaction was also used to screen for *MPL*-W515 mutations.

Results
The *JAK2*-V617F mutation was present in 11 patients (48%) and was associated with significantly higher erythrocyte and white blood cell counts (p=0.009 and 0.011, respectively). In 6/11 RARS-T patients the allelic ratio of *JAK2*-V617F was above 50%, indicating the presence of cells homozygous for the mutation. In two of these patients a transition from *JAK2*-V617F heterozygosity to homozygosity was documented and was accompanied by rising platelet counts in sequential samples. The *MPL*-W515L mutation was detected in one *JAK2*-V617F negative patient. The relative risk of death was found to be lower in the mutation-positive group than in the mutation-negative group.

Conclusions
RARS-T patients with *JAK2*-V617F have a more favorable prognosis than those without the *JAK2* mutation. The prevalence of homozygous *JAK2*-V617F mutation in RARS-T suggests that this entity is biologically distinct from essential thrombocythemia.

Key words: RARS-T, myelodysplastic syndrome, bone marrow biopsy, *JAK2* genotyping, prognosis

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Introduction

The identification of an acquired somatic mutation in the *JAK2* gene, resulting in a valine to phenylalanine substitution at position 617 (JAK2-V617F), has provided new insights into the pathogenesis of Bcr-Abl negative myeloproliferative diseases (MPD).[1-4] The *JAK2*-V617F mutation is present in most patients with polycythemia vera (PV), in about 50% of patients with essential thrombocythemia (ET) or primary myelofibrosis (PMF), but only at a low frequency in myelodysplastic syndromes (MDS).[5, 6] Among 52 patients with refractory anemia with ringed sideroblasts (RARS), a subtype of MDS, the *JAK2*-V617F allele was detected in only two cases.[7] Sideroblastic erythropoiesis is a feature of pure RARS and other subtypes of MDS,[8] but may also be associated with megakaryocytic proliferation and high platelet counts.[9-18]

Recently, the *JAK2*-V617F mutation was found in 6/9, 5/16, 3/3, 4/6, 6/12 and 5/7 RARS-T patients,[13-18] suggesting that a large proportion of RARS-T cases are *JAK2* mutation-related disorders. To further clarify this issue we assessed the V617F status of 23 patients from a large cohort of 57 patients with well-documented RARS-T by allele-specific PCR for *JAK2*-V617F genotyping.[19] We investigated the relationship between *JAK2*-V617F-positive and mutation negative subtypes with regard to morphologic, laboratory and clinical parameters and survival data. By analyzing follow-up samples we addressed the issue of whether the ratio of the mutated *JAK2* mutant allele may increase with time and disease progression.

Design and Methods

Sample collection

Following the guidelines approved by the local ethical committee, the files of the Institute of Pathology, University Hospital of Freiburg, Germany, and of the Bone Marrow Registry, University of Düsseldorf, Germany, were searched for bone marrow specimens that fulfilled the WHO criteria for RARS-T: a) evidence of >15% ringed sideroblasts (RS) on marrow smears, b) sustained high platelet counts without evidence of reactive thrombocytosis, and c) absence of defined cytogenetic abnormalities such as *BCR-ABL*, deletion 5q, inv(3)(q21q26) or t(3;3)(q21; q26). For inclusion in this study, we applied a cut-off level of 600×10^9/L. Moreover, evidence of at least erythroid dysplasia on bone marrow aspirates and biopsies was required to establish the diagnosis of RARS-T.[20] In accordance with the nomenclature recently proposed for MPD by the International Working Group for Myelofibrosis Research and Treatment, we designated cases exhibiting myelofibrosis (MF) or leukemic transformation as RARS-T MF or blast phase (BP) disease.[22]

Detection of mutations in JAK2 and MPL

DNA was isolated from fresh consecutive sections from the original diagnostic paraffin-embedded tissue blocks cut at 5µm in Eppendorf tubes. The paraffin was removed in Roti-clear (Roth, Karlsruhe, Germany; three times for 10 min at 58°C). After washing in 100% ethanol (twice for 10 min) and lysis in a digestion buffer (50 mmol Tris-HCL, 1 mmol/L EDTA, 0.5% Tween 20, and 0.5 mg proteinase K) an additional DNA purification step with NucleoSpin Blood (Macherey-Nagel, Düren, Germany) was performed. Controls included DNA from the bone marrow biopsies of three patients with PV and from 11 patients with RARS with platelet counts $\geq400\times10^9$/L but $<600\times10^9$/L.The allele-specific PCR for *JAK2*-V617F genotyping was carried out as previously described.[19] The percentages of chromosomes carrying the G>T transversion representing the *JAK2*-V617F allele (%T) were determined using a standard curve method and the formula: %T=(height of G-peak)x100. Mutations in *MPL* were screened for by allele-specific PCR using a common primer GTTTCTTCCTAGCCTGGATCTCCTTGG and fluorescently labeled primers FAM-AAACTGTAGTGTGCAGGAAACTGCA (for *MPL*-W515L) and FAM-AAAAAACTGTAGTGTGCAGGAAACTGCT (for *MPL*-W515K).

Results

Clinical and hematologic findings

The review of clinical and hematologic data identified 57 patients with RARS-T. Owing to the sometimes small size of paraffin-embedded bone marrow trephines, sufficient amounts of genomic DNA for PCR analysis could only be obtained from bone marrow trephines of 23 of a total of 57 biopsies from patients with RARS-T. The 34 patients with no amplifiable DNA did not exhibit any statistically significant differences from the 23 cases studied with regard to clinical/pathological features (p values >0.05). We can, therefore, exclude a selection bias for the patients from whom DNA was available.

The clinical, laboratory and survival data of the 23 patients with RARS-T are summarized in Table 1.

Morphological features of bone marrow smears and trephines

Bone marrow specimens typically showed hypercellularity and a disturbed topographical organization. Dyserythropoiesis was present in all patients and was characterized by large groups of immature erythroid precursors with asynchronous nuclear cytoplasmic maturation, megaloblastoid features, binucleated erythroblasts, occasional nuclear budding and karyorrhexis (Figures 1A; 2B, C, F, G). Iron stains of bone marrow aspirates consistently revealed coarse iron granules which partially or completely encircled the nuclei of >15% red cell precursors (Figures 1B; 2A, E; 3B, E, H, insets). Confluent islets of

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Table 1. Clinical, hematologic and morphological features of 28 patients with RARS-T.

Patient Nr.	Sex	Outcome	Follow-up (months)	Age at diagnosis (years)	Red blood cells (x 10^{12}/L)	Hemoglobin (g/dL)	Hematocrit (%)	MCV (fl)	Leukocytes (×10^9/L)	Platelets (×10^9/L)	LDH (U/L)	Fiber-score, initial biopsy	Fiber-score, sequential biopsy	Vascular events	Spleen	JAK2-V617F AS-PCR %T
244	M	alive	48	73	3.54	12.30	37	103	6.20	433		0			–	10
		8 m after liver biopsy			3.8	11.50	5	91	9.10	637		0			–	43
		16 m after liver biopsy			3.5	10.50	31	90	9.30	787		0	1		–	98
238	F	alive	68	69	3.13	9.00	28	89	11.30	975	240	1			–	19
		14 m after liver biopsy				9.3	27	83	13.10	1582		1			–	72
182	M	dead	58	66		5.6			4.18	1350		0			na	0
185	F	dead	63	77		9.10			11.00	1007	414	0			na	29
278	F	alive	110	74		7.4	23	85	12.50	946	335	2			+	39
224	F	dead	66	82	4.03	11.40	36	90	8.70	966	535	2	2	+	+	MPL-W515L +
239	F	alive	67	78	3.59	11.10	34	95	7.00	703		0			–	0
260	M	alive	37	66	4.65	13.50	44	95	17.80	1183		1			–	68
222	F	dead	99	72	3.55	11.60	37	104	19.50	1117		1		+	+	0
235	F	dead	65	79	3.54	11.70	34	96	6.40	747		0	0		–	0
243	F	alive	51	79	4.90	13.10	41	84	17.50	1282	396	2			+	34
514	M	alive	17	77	3.2	11.3	35	108	8.00	804	83	2			+	0
223	M	dead	23	69		10.70			5.,60	900	209	1			na	0
262	F	alive	27	77	3.53	11.20	34	96	5.50	1066	216	1			+	0
184	F	alive	129	76	3.80	10.90		87	8.30	1290		0			+	16
263	F	alive	21	75	3.71	11.30	35	95	11.26	850	281	1		+	+	79
512	F	dead	54	61	4.39	13.00	39	89	8.20	906	311	0	1	+	–	35
246	M	dead	41	75	2.50	8.10	25	101	18.45	919	325	2		+	+	0
237	M	alive	202	59	4.90	15.20			28.50	1165	418	0	1	+	+	99
511	M	dead	174	57	3.61	11.7	32		12.00	952		0	0		–	0
510	F	alive	132	65		11.40		87	5.90	707		0	3		+	78
208	F	dead	97	61	2.93	10.30	30	101	4.70	617	168	0		+	–	0
203	M	dead	5	67	2.84	9.90	29	102	4.60	1200	182	0			–	0

immature erythroblasts were easily detected by anti-hemoglobin immunolabeling of marrow sections (Figure 1C). A striking increase in megakaryocyte numbers correlated with high platelet counts up to a maximum of 1544×10^9/L. All cases included in this cohort presented with marked variability of megakaryocyte size ranging from small MDS-like forms with hypolobulated nuclei and disperse chromatin (Figures 1C, F; 2 C, H) to large more MPD-like cells with hyperlobulated nuclei (Figures 1D; E, G, H; 2D, I). Even the specimens with numerous large megakaryocytes always contained a MDS-like smaller subpopulation (Figures 1 H and J; 2C and H) in keeping with a MDS/MPD category which includes myeloid disorders that have both dysplastic and proliferative features.[23] Figure 2 clearly demonstrates that dysplastic features were not restricted to the *JAK* V617F negative patients (Figures 2A-D; patient n. 511; *JAK2* V617F 0%T) but were also present in mutated cases (Figure 3 E-I; patient n. 510; *JAK2* V617F 78%T).

The neutrophilic granulopoiesis (Figures 1H and I; naphthol AS-D chloroacetate esterase reaction) showed a wide range from mild proliferation (Figure 1H) to normal frequency or a slight decrease (Figure 1I). Dysgranulopoietic features included hypogranulation of myeloid cells, unevenly distributed granules and pseudo-Pelger forms (Figures 2B, F and G). Cases presenting with pronounced multilineage dysplasia may be referred to as refractory cytopenia with multilineage dysplasia, ringed sideroblastosis and thrombocytosis (RCMD- RS-T) as recently proposed.[24]

The blast cell count, as evaluated on bone marrow smears and on CD34 stained bone marrow sections, was initially <5% in all specimens. At initial diagnosis, various grades of reticulin fibrosis ranging from absence (score 0; Figure 1J) to slight increase in fiber density (score 1; Figure 1K) or a moderate (score 2; Figure 1L) were present in 12 patients.[25] For these cases the designation RARS-T MF was used in keeping with a recently published consensus on the terminology of MPD.[22] Sequential biopsies revealed an evolution to a higher fibrosis score in four patients, including one with a marked increase in fiber density (score 3), while no change in fibrosis was noted in three cases (Table 1).

JAK2-V617F and MPL mutation analysis

We detected the *JAK2*-V617F mutation in 11/23 of the RARS-T patients (48%) with a predominance of females (8/11 positive patients). In addition, 1/11 of RARS patients with platelet counts < 600×10^9/L was also *JAK2*-V617F positive. This patient displayed an allelic ratio of 38%T and his platelet count was borderline (596×10^9/L). In seven RARS-T patients we found an allelic ratio of <50%T at first presentation (range, 16 to 43). This finding is compatible with the presence of cells heterozygous for the *JAK2*-V617F mutation, but since we analyzed a mixture of cells, we cannot exclude the presence of a

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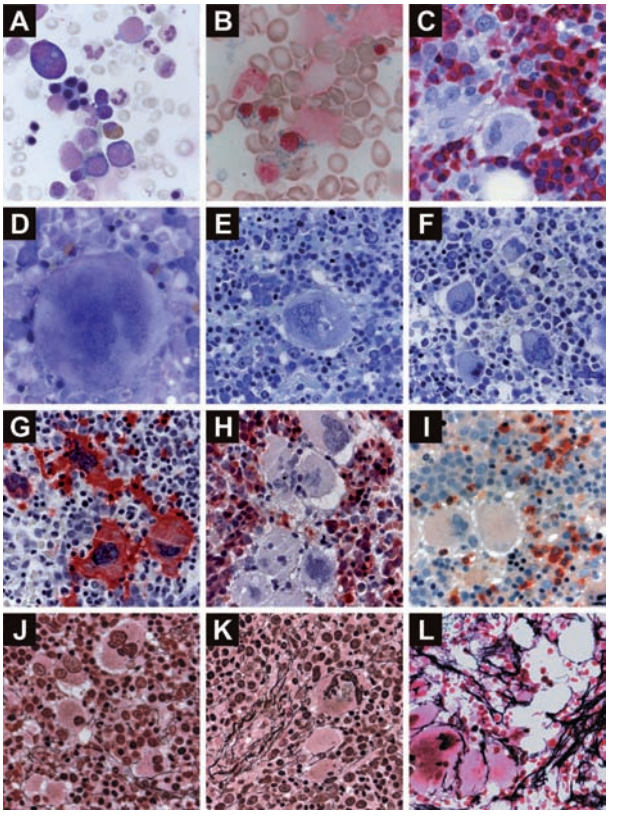

Figure 1. Bone marrow features of RARS-T: (A, B) Bone marrow aspirates with mostly immature megaloblastoid erythroblasts and cytochemically detectable iron granules (blue) encircling the nuclei of typical ringed sideroblasts with a trinucleated form in the center. (A, Pappenheim's stain; (B) Perls' stain). C. Hemoglobin immunostaining showing a predominance of erythropoiesis in a bone marrow trephine. (D,E) Giant megakaryocytes with extensive lobulation in a marrow aspirate (D, Pappenheim's stain) and biopsy (E, Giemsa). F: Evidence of dysmegakaryopoiesis with small hypolobulated dysplastic forms in a specimen classified as refractory cytopenia with multilineage dysplasia, ringed sideroblastosis and thrombocytosis (RCMD RS-T; Giemsa stain). (G) Marked increase of clustered giant megakaryocytes with hyperlobulated nuclei (G, CD61 immunostain). (H, I) Wide range of neutrophilic granulopoiesis (H,I; naphthol AS-D chloroacetats esterase reaction) showing a mild proliferation (H) or a decrease and maturation defects in rare cases of RCMD RS-T (I). (J-L) Variable amounts of reticulin from normal (J) to mild (K) or moderate (L) fibrosis consistent with RARS-T MF in K and L (J-L, silver impregnation). A-C × 1000; D-L C × 400.

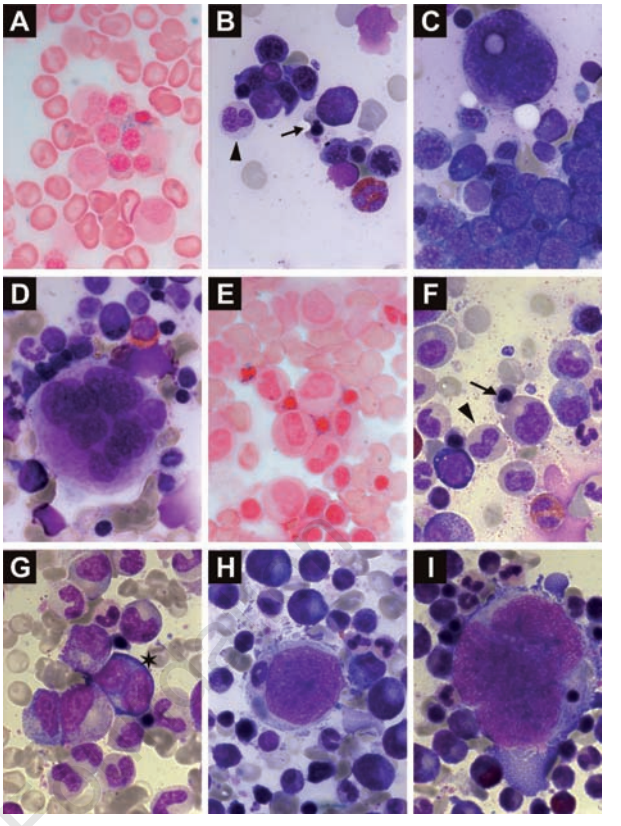

Figure 2. Bone marrow morphology demonstrating both dysplastic and proliferative features in a *JAK* V617F negative patient (n. 511; A-D) and a patient with the mutation (n. 510; E-I). Ringed sideroblastosis (A,E) associated with immaturity, megaloblastoid changes and abnormal nuclear budding (arrows) and binuclearity (asterix) of erythroblasts (B,C,F,G). Dysgranulopoiesis with numerous hypogranular (arrowheads) myeloid cells (B,F,G; Pappenheim's stain). Evidence of both small megakaryocytes with round nuclei and mature cytoplasm (C,H) and large multinucleated forms (D, I). A, E, Perls' stain; B-D, F-I, Pappenheim's stain; × 1000.

small population of cells that were already homozygous for *JAK2*-V617F. In the remaining four cases, the mutant allele predominated (>50%T), demonstrating that cells homozygous for *JAK2*-V617F were present. Interestingly, in two patients with initially low allelic ratios for *JAK2*-V617F, we observed an increase of the allelic ratio in the follow-up samples accompanied by an increase in platelet counts. In patient n. 244, the allelic ratio at initial diagnosis was 10%T with a platelet count of 433×10^9/L, thus meeting the diagnostic criteria of RARS, but not of RARS-T. After 8 months of follow-up the allelic ratio of mutated *JAK2* increased to 43%T and the platelet count rose to 637×10^9/L, i.e. progressing by definition to RARS-T, and at 16 months to 98%T with a platelet count of 787×10^9/L (Table 1 and Figure 3A, D and G). This molecular evolution was also reflected by a progression in the morphological disease features in sequential bone marrow biopsies, especially an increase in megakaryopoiesis. Contrasting with the presence of loosely distributed normal-sized megakaryocytes in the RARS phase (Figure 3B, C), an increase in megakaryocytes and an atypical clustering accompanied the transition into RARS-T and later RARS-T MF was paralleled by an increase of the *JAK2*-V617F allelic ratio in specimens taken 8 months (Figure 3E, F) and 16 months after the initial sample (Figure 3 H. I). In patient n. 238, the *JAK2*-V617F allelic ratio increased from 19% to 72% within 14 months, also paralleled with an increase in the platelet count (Table 1). Thus, at last follow-up 6/11 RARS-T patients had an allelic ratio >50%T and evidence of the presence of cells homozygous for *JAK2*-V617F (Figure 4). A third patient from whom sequential biopsies were available remained negative for the *JAK2* mutation over a period of 3 years. In patient n. 224, we detected the *MPL*-W515L mutation (*not shown*). This patient was negative for *JAK2*-V617F and presented with grade 2 fibrosis. We did not detect either *MPL*-W515L or *MPL*-W515K mutations in any other patient with RARS-T. The sensitivity of our allele specific-PCR assay is <1%.

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Table 2. Clinicopathological correlations in *JAK2*-V67F positive *vs* negative RARS-T.

	JAK2V617F	*JAK2 wild-type*	*p-value*
Number	11 (48%)	12 (52%)	
Female	8 (73%)	6 (50%)	0.40
Age (years)	70.4±6.8	71.9±7.4	0.48
Hemoglobin (g/dL)	11.4±2.3	10.4±1.8	0.31
Red blood cells ($\times 10^{12}$/L)	4.16±0.64	3.33±0.46	0.009
Mean corpuscular volume (fL)	89.1±3.9	99.2±5.5	0.002
Leukocytes ($\times 10^{9}$/L)	12.9±6.4	7.8±4.3	0.011
Platelets ($\times 10^{12}$/L)	995±218	845±214	0.56
Myelofibrosis*	9 (82%)	6 (50%)	0.19

**in initial and/or sequential biopsies.*

Correlation of the JAK2-V617F mutation status with clinico-pathological features

JAK2 mutation status had no impact on platelet count (p=0.56), spleen size (p=1.00), marrow fibrosis (p=0.19) or all vascular events (p=0.67). The JAK2-V617F positive patients had significantly higher red blood cell counts (p=0.009), lower mean corpuscular volume (MCV) (p=0.002) and higher leukocyte count (p=0.011; Table 2). In initial and/or sequential bone marrow trephines, 9/11 *JAK2*-V617F positive and 6/12 negative patients fitted in an early or manifest fibrotic stage that we designated as RARS-T MF (p=0.19). An evolution into BP occurred in two *JAK2*-V617F negative patients, but in none of the positive patients. The relative risk of death for the *JAK2*-V617F-positive patients was 79% lower than that of the negative group (relative risk of *JAK2*-V617F positive versus negative 0.205; 95% confidence interval: 0.044-0.958; p=0.044) paralleled by 5-year survival probabilities of 86% *vs.* 62% (Figure 5).

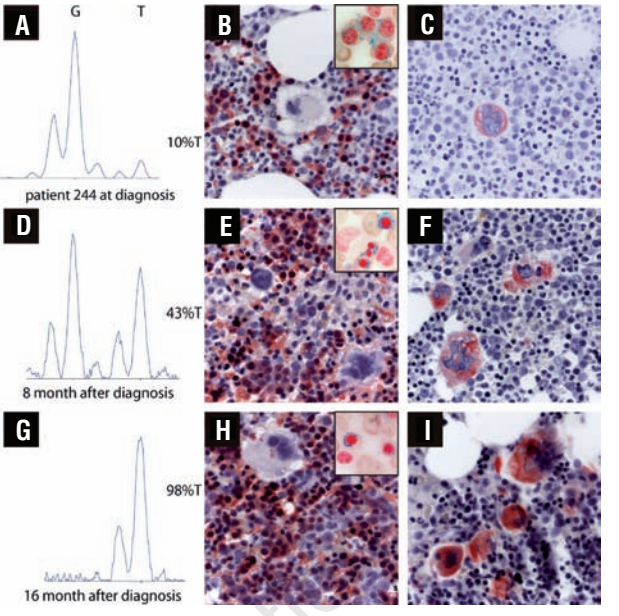

Figure 3. Molecular and morphologic findings in patient n. 244 at initial diagnosis (A-C) and in sequential biopsies 8 (D-F) and 16 (G-I) months later. Characteristic chromatograms of DNA samples obtained from sequential biopsies demonstrate the increase of the allelic ratio of mutated *JAK2* from 10%T to 43%T and than to 98%T (A, D, G). A moderate erythroid hyperplasia with mild megaloblastoid maturation defects and cytochemically detectable iron granules (blue) encircling the nuclei of typical ringed sideroblasts (inserts, bone marrow smears) is a common feature of the three biopsies (B, E, H; immunolabeling for hemoglobin; inserts, Perls' stain). The increase in megakaryocyte number, atypia and clustering is evident on CD61 immunostains (C, F, I).

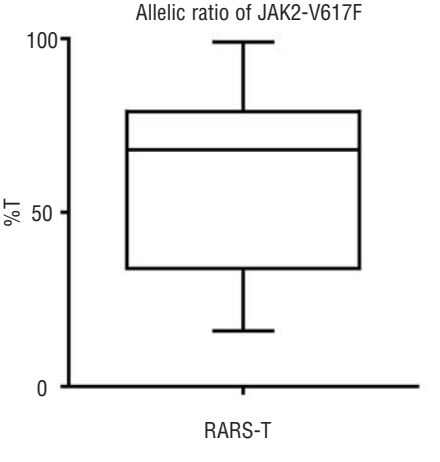

Figure 4. Allelic ratio between the mutant and the wild-type *JAK2* allele (%T) in RARS-T. At last follow-up 6/11 RARS-T patients had an allelic ratio >50%T (mean 59%).

Discussion

Our data support the concept that RARS-T shares several of the distinct pathophysiological features common to classic Philadelphia-chromosome negative MPD including striking megakaryocytic proliferation, overproduction of platelets, vascular events, and abnormalities of chromosomes 8 and 20 and MF.[26] These features may be driven by activated JAK2 signaling in a subset of RARS-T. However, the question arises of which molecular mechanisms may associate the *JAK2* mutation with anemia in RARS-T, while it confers a multilineage proliferative advantage in other MPD. The higher age of our patients with RARS-T (median age 71 years) compared to that of patients with either typical ET or PMF further supports the concept that the typical MPD/MDS phenotype may be determined by the acquisition of a variety of genetic and/or epigenetic changes. Mitochondrial DNA mutations leading to ringed sideroblastosis and up-regulation of mitochondrial-related genes in $CD34^{+}$ cells have been demonstrated in patients with RARS-T.[27-29] Recently, ineffective erythropoiesis and high levels of apoptosis have been ascribed to not yet explained molecular alterations in RARS-T which may abolish the effect of JAK2 activation.[18]

However, we observed that in absence of iron deficiency the erythrocyte counts were significantly higher and the MCV lower in the *JAK2*-V617F positive group compared to in patients with wild type *JAK2*. This find-

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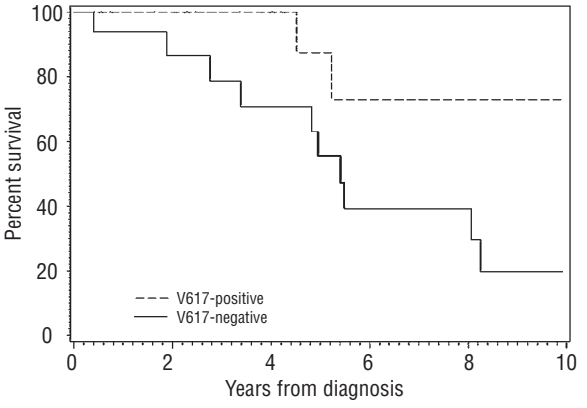

Figure 5. Kaplan-Meier-survival analysis of patients with RARS-T according to *JAK* V617F-mutation status. The survival probability is significantly better for patients with the mutation than for those without.

ing suggests that *JAK2*-V617F may at least partially overcome the mechanisms which induce the megaloblastoid maturation defects of sideroblastic erythropoiesis in RARS-T. In ET, the mutation-positive subgroup was found to have lower MCV than the subgroup without the mutation and contained more patients with MCV values below the normal range.[30] As previously reported for ET,[30] *JAK2*-V617F positive patients in our cohort had significantly higher white cell counts in keeping with a proliferative signal of *JAK2*-V617F to the myeloid lineage in RARS-T. The incidence of *JAK2*-V617F in our series (11/23 cases; 48%) fits well in the range reported in previous studies of RARS-T.[13-18]

All patients included in our study presented with a spectrum of concomitant myeloproliferative and dysplastic features in keeping with a mixed MDS/MPD phenotype.[23] Cases presenting with diagnostic criteria of classical ET or PMF associated with RS without overt dysplastic changes were excluded from the study.

Most studies on the *JAK2*-V617F mutation in RARS-T did not provide information concerning the *JAK2* allelic ratio or the progression in patients with RARS-T.[13,14,16-18] However, since the large majority of RARS patients, including our control group with RARS, do not carry the mutation, the occurrence of *JAK2*-V617F on the background of RARS is obviously a rare event. One of the most interesting aspects of our work is the association between the activating *JAK2*-V617F mutation and rising platelet counts in RARS-T. The progression in the allelic ratio from low heterozygous to high homozygous *JAK2*-V617F was paralleled by an increasing thrombocytosis in both *JAK2*-V617F positive patients from whom sequential biopsies were available. Our observation suggests that a deregulation of *JAK2* may precede the development of typical RARS-T. In a recently published series of RARS-T, *JAK2*-V617F was amplified from a bone marrow smear of a patient with normal platelet counts who apparently developed RARS-T 5 years later.[15] No follow-up information concerning the allelic ratio of *JAK2* of this patient was available. The range of allelic ratios in all *JAK2*-V617F positive patients was 4 to 35%T suggesting heterozygosity, while the range in our patients was compatible with the presence of a homozygous *JAK2*-V617F mutation in six patients (21.4%).[15] Thus, with regard to the allele burden, RARS-T differs from true ET, which reportedly never or rarely harbors the mutation in a homozygous status.[30,31]

The current data concerning the prognostic relevance of *JAK2*-V617F in ET and PMF are still inconclusive.[32,33]

Our data suggest that RARS-T patients positive for *JAK2*-V617F have a more favorable prognosis than patients without the *JAK2* mutation. Previously, it has been shown that the survival of RARS-T patients as a whole is shorter than that of patients with typical ET.[16,21] Here we provide evidence that better survival probability is significantly associated with *JAK2*-V617F mutation status. The relative risk of death of the *JAK2* mutation-positive patients was 79% lower than that of the negative group, with 5-year survival probabilities of 86% *vs* 62%. The inferior survival of the JAK2 mutation-negative patients may be due to the predominance of dysplastic features reflected by lower erythrocyte and leukocyte counts, higher MCV and even multilineage dysplasia of the bone marrow in a subset of patients. Until now, RARST BP was observed only in *JAK2*-V617F negative patients.

In our cohort, *JAK2*-V617F positive RARS-T patients shared many clinical, laboratory and morphological features with negative patients. Therefore, it seems likely that similar downstream pathways may be activated by different mediators of signal transduction in the *JAK2*-V617F negative subtypes of RARS-T. Our results show that an acquired mutation of the thrombopoietin receptor, *MPL*-W515L, may also be involved in the pathogenesis of RARS-T, at least in some cases.

In conclusion, the link between *JAK2*-V617F mutational status and prognosis, as well as the transition from heterozygosity to homozygosity and the relatively high frequency of a homozygous status that we found in our patients indicate that RARS-T displays distinct clinicopathological and molecular features which are different from those of classical ET and PMF. A challenging observation is the presence of an allelic T:G ratio of 99% in one patient suggesting that nearly all bone marrow cells carried two copies of mutant *JAK2*. It may, therefore, be assumed that a homozygous mutation can occur in a multipotent hematopoietic stem cell in some RARS-T patients. Since hematopoietic cells that are homozygous for *JAK2* V617F are absent in most cases of ET, this finding further suggests that RARS-T does not just represent a variant form of ET and should be distinguished from the category ET-RS. The pathogenesis of RARS-T appears to involve, at least in part, *JAK2*-V617F independent molecular events, which remain to be further characterized.

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Authorship and Disclosures

AHSG and RCS conceived and designed the study and wrote the paper; SST, FS and RCS performed the molecular research; ASG, AK, SH, PR and UG collected data and analyzed bone marrow and blood samples; SH contributed to figure preparation; MO performed the statistical analyses.

The authors reported no potential conflicts of interest.

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