Int J Hematol (2008) 88:387–395
DOI 10.1007/s12185-008-0169-1

ORIGINAL ARTICLE

Refractory anemia with ringed sideroblasts associated with thrombocytosis: comparative analysis of marked with non-marked thrombocytosis, and relationship with JAK2 V617F mutational status

J. M. Raya · L. Arenillas · A. Domingo · B. Bellosillo · G. Gutiérrez · E. Luño · M. A. Piñán · M. Barbón · M. L. Pérez-Sirvent · M. J. Muruzábal · L. Yánez · L. García · A. Lemes · J. T. Navarro · A. Elosegi · M. A. Cortés · A. Villegas · M. A. Durán · M. Ardanaz · L. Florensa · On behalf of the Grupo Español de Citología Hematológica (GECH), Working Group into the Asociación Española de Hematología y Hemoterapia (AEHH)

Received: 26 March 2008 / Revised: 3 August 2008 / Accepted: 27 August 2008 / Published online: 27 September 2008

Abstract The World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues (2001) defined a provisional entity named refractory anemia with ringed sideroblasts associated to marked thrombocytosis (RARS-MT). Diagnosis of RARS-MT requires more than 15% of ringed sideroblasts in bone marrow aspirate and the existence of a thrombocytosis in blood, with a platelet count above 600×10^9/L. Nevertheless, controversy exists regarding this platelet count "cut-off" value and, when RARS-MT was defined, the JAK2 mutation and its importance in the study of myeloproliferative disorders was unknown. We present the results of a Spanish retrospective multicentric study, which includes 76 cases of RARS with associated thrombocytosis (platelet count above 400×10^9/L) at diagnosis (RARS-T), 36 of them with a platelet count above 600×10^9/L. Our aim was to analyze their clinical, analytical and morphological characteristics, and to establish correlations with the JAK2 mutational status.

Keywords Refractory anemia · Ringed sideroblasts · Thrombocytosis · JAK2 mutation

1 Introduction

The World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues, in the chapter dedicated to myelodysplastic/myeloproliferative disease, unclassifiable (category MDS/MPD, U), defines a provisional entity named refractory anemia with ringed sideroblasts associated to marked thrombocytosis (RARS-MT) [1]. Diagnosis of RARS-MT requires more than 15% of ringed sideroblasts in bone marrow aspirate and the existence of a thrombocytosis in blood, with a platelet count above 600×10^9/L. In these patients coexist features of myeloproliferative disease and myelodysplastic syndrome. The experts' panel considered that the term "unclassifiable" was necessary until future studies indicate a more exact classification, and three possibilities were suggested: RARS-MT as a distinct entity, one group in the spectrum of RARS, or the simultaneous occurrence of two separate disorders (RARS and essential thrombocythemia). At that moment, there was no evidence of cytogenetic or molecular genetic alterations specific for this group, and it was mandatory to exclude in these patients the presence of the Philadelphia chromosome and the *BCR/ABL* fusion gene, as well as a 5q- syndrome and abnormalities of chromosome 3q21q26.

The Spanish Group of Hematologic Cytology (*Grupo Español de Citología Hematológica*, GECH) proposed 2 years ago to develop a national survey of those cases diagnosed of RARS, in which an associated thrombocytosis

This work is multicentric, with data collected and centralized at Hospital Universitario de Canarias (La Laguna, Spain).

J. M. Raya (✉) · L. Arenillas · A. Domingo · B. Bellosillo · G. Gutiérrez · E. Luño · M. A. Piñán · M. Barbón · M. L. Pérez-Sirvent · M. J. Muruzábal · L. Yánez · L. García · A. Lemes · J. T. Navarro · A. Elosegi · M. A. Cortés · A. Villegas · M. A. Durán · M. Ardanaz · L. Florensa
Servicio de Hematología y Hemoterapia,
Hospital Universitario de Canarias,
Ofra, s/n, 38320 La Laguna, Tenerife, Spain
e-mail: jomaraya@yahoo.es

(platelet count above 400 $\times$ 10^9/L) was found at diagnosis, with the aim to analyze their clinical, analytical and morphological characteristics. In the last 2 years, our group has reported partial results concerning this study in different national and international Hematology congresses [2].

2 Patients and methods

2.1 Sample collection

We retrospectively studied 76 patients with diagnosis of RARS and concomitant thrombocytosis (RARS-T), obtained from a national survey with the participation of 19 Spanish hospitals. Those cases with a clear initial diagnosis of essential thrombocythemia without associated anemia were not included in the present study, even when a bone marrow ringed sideroblasts percentage was above 15% in any moment of their clinical evolution. Of the 76 analyzed cases, 36 fulfilled the WHO diagnostic criteria for RARS-MT (platelet count >600 $\times$ 10^9/L), while 40 patients presented with a "non-marked" thrombocytosis (400–600 $\times$ 10^9/L, RARS-nMT). A data collecting page, previously designed, was distributed to the participating hospitals. Analyzed data included age at diagnosis, sex, motive for initial consultation, presence or absence of splenomegaly, hemogram (with special attention to white blood cell count, basophil count, hemoglobin level, platelet count and mean corpuscular volume, MCV), presence or absence of leukoerythroblastosis, leukocyte alkaline phospatase index, biochemical parameters (uric acid; lactate-dehydrogenase (LDH); ferritin; vitamin B12), karyotypic study, presence or absence of JAK2 V617F mutation, associated complications (non-hematologic neoplasms, evolution to acute leukemia, thrombotic or bleeding events, evolution to myelofibrosis), therapeutic attitude (watch and wait, cytorreduction-inducer drugs, oral antiagregant agents, transfusional support), time from diagnosis, and survival if death occurred.

2.2 Morphology features of blood and bone marrow specimens

A centralized review of morphological findings in peripheral blood and bone marrow aspirate smears at diagnosis was performed in 43 out 76 patients. In peripheral blood, we studied the following morphological parameters: dyshemopoietic features in each lineage, percentage of basophils, percentage of blast cells and presence or absence of leukoerythroblastosis. In bone marrow aspirate specimens, together with percentage of ringed sideroblasts, percentage of type-III sideroblasts, and iron storages in mononuclear phagocytic system (Perls' stain), we also evaluated marrow cellularity, presence or absence of mastocytes, macrophagues, sea-blue histiocytes and apoptotic figures, percentage and type of erythroid, myeloid, and megakaryocytic dysplasia and percentage of blast cells (type I and II). Bone marrow biopsy examination included cellularity, quantity of megakaryocytes and reticulin fibrosis degree.

2.3 Analysis of JAK2 mutational status

DNA was extracted from peripheral blood or bone marrow smears of 47 patients at diagnosis. Allele-specific PCR for the detection of JAK2-V617F was performed on a ABI 7900 HT real time PCR system (Applied Biosystems) using specific Taqman primers for the mutated allele and for the non-mutated sequence as previously described [3].

2.4 Statistical analysis

Numerical variables are expressed with mean and standard deviations. Categorical and ordinal variables are expressed with frequencies and percentages. Features of patients grouped according to platelet count and JAK2 mutational status were compared using Mann–Whitney test. Proportions between groups were compared with Chi-squared test or Fisher's exact test as appropriate. Multivariable analysis was not recommended because of the relative small size of the sample for such purpose. Cox proportional hazard regression model was used to compare survival times and Kaplan–Meier curves to represent cumulative survival. All tests were two-sided and P values <0.05 were considered statistically significant. Statistical analyses were performed using SPSS (version 14.0.1, Chicago, Ill., USA).

3 Results

Mean age of the whole group of patients was 72.8 years (SD 10.3, range 29–89) and 42 men and 34 women were included. Most frequent form of presentation was casual finding (44.4%) or anemic syndrome (42.9%), and less often a constitutional syndrome (7.9%), a thrombotic event (3.2%) or a bleeding episode (1.6%). Mean follow-up of patients was 46.0 $\pm$ 36.1 months (range 1–174), and 23 patients (30.3%) have died at the moment of closing this study.

There were no differences between RARS-MT and RARS-nMT patients (Table 1) regarding age (73.2 $\pm$ 8.9 vs. 72.4 $\pm$ 11.5) and sex (21 male and 15 female patients in the first group and 21 male and 19 female patients in the second group). Although all the patients had anemia at diagnosis (considered when hemoglobin levels were

Table 1 Main clinical and analytical features of patients with RARS-T, comparing RARS-MT and RARS-nMT groups at diagnosis

	RARS-MT ($n = 36$)	RARS-nMT ($n = 40$)	P value
Age (years)	73.2 ± 8.9	72.4 ± 11.5	NS
Sex (M:F)	21:15	21:19	NS
Platelet count ($\times 10^9$/L)	898 ± 282	477 ± 54	<0.001
WBC count ($\times 10^9$/L)	9.0 ± 3.6	6.7 ± 2.3	0.005
Hemoglobin (g/L)	104.4 ± 18.3	100. 1 ± 14.2	0.06
MCV (fL)	98.1 ± 7.3	104.1 ± 5.8	0.001
Basophil count ($\times 10^9$/L)	0.103 ± 0.093	0.074 ± 0.083	NS
Bone marrow blasts (%)	1.0 ± 1.2	1.0 ± 1.1	NS
Type III sideroblasts (%)	26 ± 21	23 ± 19	NS
Ringed sideroblasts (%)	44 ± 20	49 ± 17	NS
Uric acid (mg/dL)	5.7 ± 2.3	5.6 ± 1.5	NS
Lactate-dehydrogenase (U/L)	415 ± 213	352 ± 130	NS
Ferritin (ng/mL)	643 ± 934	349 ± 233	NS
Vitamin B_{12} (pg/mL)	753 ± 662	700 ± 433	NS
Splenomegaly	10/32 (31%)	2/32 (6%)	0.011
Karyotype aberrations	4/24 (17%)	3/30 (10%)	NS
JAK2 mutation	14/23 (61%)	3/24 (12.5%)	0.001
BM Megakaryocytic hyperplasia	16/20 (80%)	4/9 (45%)	0.07
BM reticulin fibrosis	9/15 (60%)	2/10 (20%)	0.06
Transfusional dependence	7/30 (23%)	11/36 (31%)	NS

Data expressed as mean ± standard deviation (continuous variables) or frequencies − percentages (categorical variables)

NS Not significant, *BM* bone marrow

<125 g/L in women and <135 g/L in men), incidentally discovered anemia was the main reason for consultation in the group of RARS-MT (46.7%), while anemic symptomatology was predominant among patients with RARS-nMT (48.5%). Patients with RARS-MT had significantly higher WBC count ($P = 0.005$) and higher incidence of splenomegaly ($P = 0.011$), while patients with RARS-nMT had higher MCV ($P = 0.001$), and a trend to lower hemoglobin levels ($P = 0.06$). Leukocyte alkaline phospatase index showed high values in the majority of patients with marked thrombocytosis (58.3%) when compared to patients with not-marked thrombocytosis; in the latter group its value was in the normal range in higher number of cases (58.8%) ($P = 0.021$). Platelet count had no relation with basophil count; percentage of marrow blasts, type-III sideroblasts, ringed sideroblasts, and iron storages in mononuclear phagocytic system (Fig. 1a, b); plasmatic levels of uric acid, LDH, ferritin or vitamin B12.

In peripheral blood, we observed the presence of erythrocytes with the coexistence of basophilic stippling and abnormal hemoglobin distribution in 84% of samples. Seventy-five percent of smears revealed the presence of dacriocytes, but only 15% presented a large number of them. Dysgranulopoiesis was a rare event, found in less than 5% of cases. The presence of large platelets was seen in 40% of smears, but hypo/agranulated was less common (only in 10%). On bone marrow aspirates evaluation, we noticed a light increase of global cellularity without a complete disappearance of the fat. All the patients displayed a high number of macrophagues and, some of them were of sea-blue histiocyte type. Almost 60% of patients had an increase in the number of normal mastocytes. All the samples displayed hyperplasia of megakaryocytes (Fig. 1c), either dispersed or in loose clusters of three or more cells. Thirty-two of the 43 samples showed dysmegacaripoiesis in 10–50% of the elements: megakaryocytes with mono-, bi-lobulated or with multiple widely-separated nuclei, micromegakaryocytes (only in one patient) and giant forms with deeply hiperlobated nuclei. Diserithropoiesis was a constant phenomenon: erythroblasts with basophilic stippling and abnormal hemoglobin distribution, asynchronous nuclear cytoplasmic maturation, megaloblastoid changes (Fig. 1d) and, rarely, internuclear bridging. We observed light dysplasia of granulopoietic lineage and only two patients showed hipogranularity with pseudo Pelger-Huet anomaly. The number of blast cells was always lower than 5%. We could not correlate any of these findings with the mutational status of JAK2.

Although only performed in 38% of patients, bone marrow biopsy showed a trend toward a more intense megakaryocytic hyperplasia (Fig. 1e) and reticulin fibrosis in patients with RARS-MT ($P = 0.07$ and $P = 0.06$, respectively); hypercellularity was found in most samples from both the groups. Prevalence of chromosome aberrations was similar in both groups of patients (16.7 vs. 10%, respectively). The following karyotypic anomalies were detected in four different patients of the RARS-MT group,

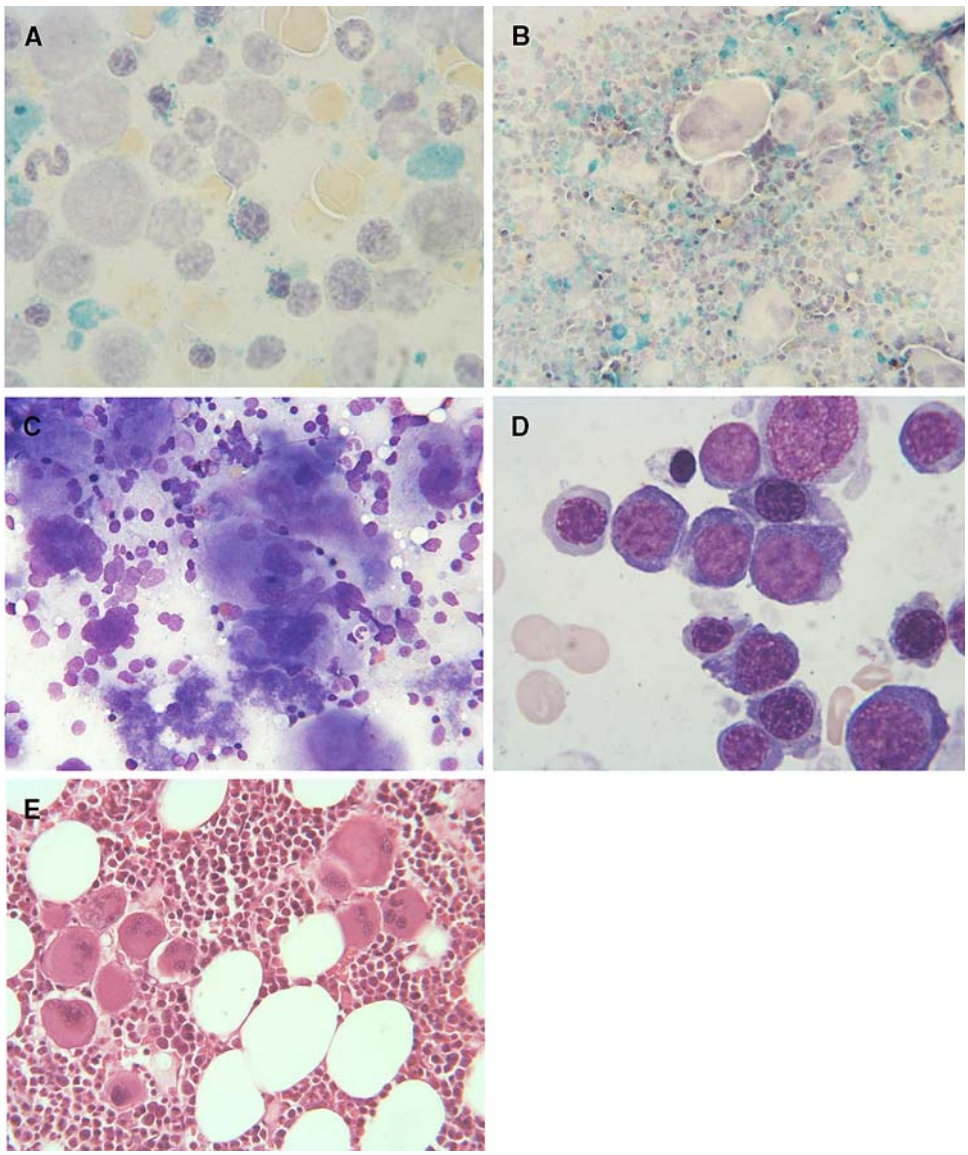

Fig. 1 Bone marrow features of RARS-T. **a** Typical ringed sideroblasts with iron granules encircling the nuclei (Perls' stain, ×1000). **b** Significant increment of iron storages in mononuclear phagocytic system (Perl's stain, ×400). **c** Marked megakaryocytic hyperplasia and occasional platelet aggregates (May–Grünwald–Giemsa, ×400). **d** Erythroid hyperplasia and precursors with megaloblastoid features (May–Grünwald–Giemsa, ×1,000). **e** Bone marrow trephine biopsy showing a marked proliferation of megakaryocytes with a tendency to loose clusters distribution (Hematoxilin–eosin, ×100)

del(11)(q21), del(20)(q12), dup(1)(q23q32), and −Y. Chromosome alterations in three cases of RARS-nMT included del(5)(q12q33), trisomy 8 and −Y. In none of the whole series, the *BCR/ABL* fusion gen or the isolated 5q- cytogenetic anomaly was detected. The analysis of JAK2 mutational status was achieved in 44/77 patients (62%) and clear differences were found: 14/23 patients with RARS-MT were carriers of the JAK2 V617F mutation (61%), whereas only 3/24 patients (12.5%) in the group of RARS-nMT ($P = 0.001$).

From a point of view of eventual complications in disease evolution, there were no differences between RARS-MT and RARS-nMT groups. Evolution to acute leukemia occurred in one patient with RARS-MT and in two with RARS-nMT; non-hematologic neoplasms appeared in the follow-up in three patients with RARS-MT and in two cases of RARS-nMT; myelofibrotic transformation of bone marrow was observed in two patients with RARS-MT and in only one with RARS-nMT (unfortunately, the analysis of the JAK2 mutational status was not possible in these three last patients). There were no differences between both groups of patients related to thrombotic complications (1 in 28 analyzed cases of RARS-MT and 2 in 31 RARS-nMT) or bleeding episodes (4 in 29 RARS-MT vs. 3 in 30 RARS-nMT). Revision of therapeutic approach showed that "watch and wait" was the most frequent strategy applied in both the groups (64.5 and 80%, respectively), although 29% of patients with RARS-MT eventually needed hydroxyurea, and only in 14.3% of patients with RARS-nMT needed hydroxyurea. Oral antiagregation was indicated in 32.1% in the RARS-MT group and in 19.4% of RARS-nMT. Packed red cell transfusion as usual management practice was similar in both groups (23.3% *versus* 30.6%). Finally, survival rates were also equivalent (Fig. 2).

The presence of JAK2 mutation in three patients with a platelet count between 500 × 10^9/L and 600 × 10^9/L led us to compare the features of the patients considering 500 × 10^9/L as new "cut-off" point (Table 2). In this analysis, the majority of previously observed differences (with a platelet count of 600 × 10^9/L as "cut-off") disappeared". Only two variables were clearly different: a significantly higher incidence of the JAK2 mutation in patients with platelets >500 × 10^9/L (53 vs. 0%, $P < 0.001$) and a more frequent presence of megakaryocytic hyperplasia in bone marrow trephine biopsy samples in the same group of patients (83 vs. 17%, $P = 0.005$). The survival rates persisted similar.

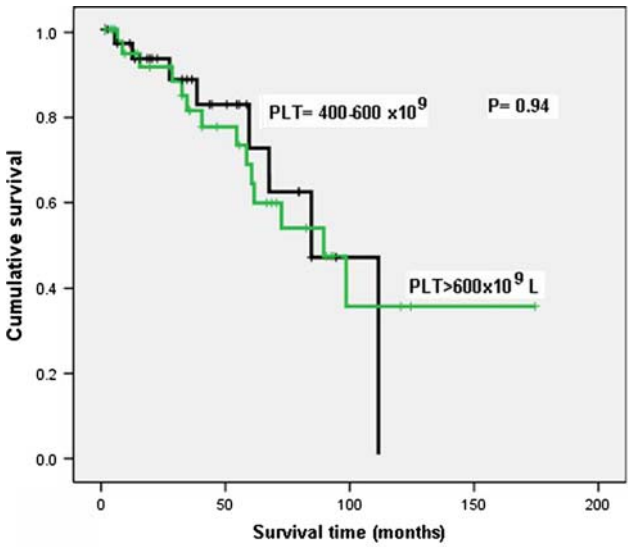

Fig. 2 Kaplan–Meier survival curves of patients with diagnosis of RARS-MT and RARS-nMT

The correlation of the JAK2 V617F mutation status with clinicopathological features of patients with RARS-T was also analyzed (Table 3). Patients with the JAK2 mutation had significantly higher platelet ($P < 0.001$) and WBC count ($P = 0.018$). On the other hand, patients without this mutation had lower hemoglobin levels ($P = 0.029$) and higher MCV ($P = 0.037$). Splenomegaly was found in 29% of patients carrying the JAK2 mutation, and in 13% of patients without this mutation. In our study, JAK2 mutation had influence neither on age, sex, peripheral blood basophil count, leukocyte alkaline phospatase index, percentage of blasts in bone marrow, percentage of type-III sideroblasts nor on percentage of ringed sideroblasts, plasmatic levels of uric acid, LDH, ferritin and vitamin B12, and frequency of karyotypic aberrations or incidence of associated complications (non-hematologic neoplasms, evolution to acute leukemia or myelofibrosis, and thrombotic or bleeding events). In our short series of bone marrow biopsied patients, those with JAK2 mutation had higher prevalence of myelofibrosis at diagnosis (71 vs. 40%). From a therapeutical point of view, retrospectively analyzed, policy was clearly different: in patients without JAK2 mutation,

Table 2 Main differences among patients with RARS-T when a platelet count of 500 × 10^9/L is considered as cut-off value at diagnosis

	RARS-T Plt > 500 × 10^9/L ($n = 48$)	RARS-T Plt < 500 × 10^9/L ($n = 28$)	P value
Age (years)	73.0 ± 11.4	72.4 ± 8.2	NS
Sex (M:F)	26:22	16:12	NS
Platelet count (×10^9/L)	810 ± 289	448 ± 28	<0.001
WBC count (×10^9/L)	8.2 ± 3.5	7.1 ± 2.5	NS
Hemoglobin (g/L)	102.7 ± 16.9	101. 0 ± 15.5	NS
MCV (fL)	100.2 ± 7.8	102.9 ± 5.4	NS
Basophil count (×10^9/L)	0.090 ± 0.084	0.083 ± 0.096	NS
Bone marrow blasts (%)	1.0 ± 1.2	0.9 ± 1.1	NS
Type III sideroblasts (%)	29 ± 22	18 ± 14	NS
Ringed sideroblasts (%)	44 ± 18	53 ± 18	NS
Uric acid (mg/dL)	5.5 ± 2.2	6.1 ± 1.2	NS
Lactate-dehydrogenase (U/L)	400 ± 197	349 ± 122	NS
Ferritin (ng/mL)	563 ± 820	356 ± 243	NS
Vitamin B_{12} (pg/mL)	700 ± 582	768 ± 483	NS
Splenomegaly	10/42 (24%)	2/22 (9%)	NS
Karyotype aberrations	6/33 (18%)	1/21 (5%)	NS
JAK2 mutation	17/32 (53%)	0/15 (0%)	<0.001
BM Megakaryocytic hyperplasia	19/23 (83%)	1/6 (17%)	0.005
BM reticulin fibrosis	9/18 (50%)	2/7 (29%)	NS
Transfusional dependence	9/41 (22%)	9/25 (36%)	NS

Data expressed as mean ± standard deviation (continuous variables) or frequencies – percentages (categorical variables)

NS Not significant, *BM* bone marrow

Table 3 Influence of the presence or absence of the JAK2 V617F mutation in the clinical picture of patients with RARS-T

	RARS-T with JAK2 V617Fmutation ($n = 17$)	RARS-T without JAK2 V617F mutation ($n = 30$)	P value
Age (years)	72.9 ± 11.1	73.6 ± 8.8	NS
Sex (M:F)	11:6	17:13	NS
Platelet count ($\times 10^9$/L)	845 ± 278	591 ± 239	<0.001
WBC count ($\times 10^9$/L)	9.2 ± 3.5	6.7 ± 2.2	0.018
Hemoglobin (g/L)	109.2 ± 16.6	97.7 ± 16.0	0.029
MCV (fL)	97.3 ± 8.7	102.4 ± 6.4	0.037
Basophil count ($\times 10^9$/L)	0.108 ± 0.082	0.063 ± 0.060	NS
Bone marrow blasts (%)	0.9 ± 1.2	1.2 ± 1.2	NS
Type III sideroblasts (%)	31 ± 27	32 ± 23	NS
Ringed sideroblasts (%)	48 ± 21	48 ± 19	NS
Uric acid (mg/dL)	5.3 ± 2.4	5.8 ± 1.8	NS
Lactate-dehydrogenase (U/L)	429 ± 191	324 ± 106	NS
Ferritin (ng/mL)	656 ± 674	560 ± 984	NS
Vitamin B_{12} (pg/mL)	579 ± 391	677 ± 577	NS
Splenomegaly	4/14 (29%)	3/23 (13%)	NS
Karyotype aberrations	2/15 (13%)	2/22 (9%)	NS
BM Megakaryocytic hyperplasia	7/8 (87%)	4/5 (80%)	NS
BM reticulin fibrosis	5/7 (71%)	2/5 (40%)	NS
Transfusional dependence	1/14 (7%)	8/27 (29%)	NS

Data expressed as mean ± standard deviation (continuous variables) or frequencies − percentages (categorical variables)

NS Not significant, *BM* bone marrow

abstention was the standard approach (89%), while it was adopted in 50% of carriers of the JAK2 mutation. Hydroxyurea as cytoreductor agent was used in 42.9% of patients carrying the mutation, while it was only sporadically used (3.7%) in patients without the mutation ($P = 0.012$). Although statistically not significant, packed red cell transfusion requirement was higher in patients without the JAK2 mutation (29%) when compared with carriers of the JAK2 mutation (7%). The same was detected when patients were grouped according to platelet count: survival rates were similar in JAK2-mutated and JAK2-non mutated groups (Fig. 3).

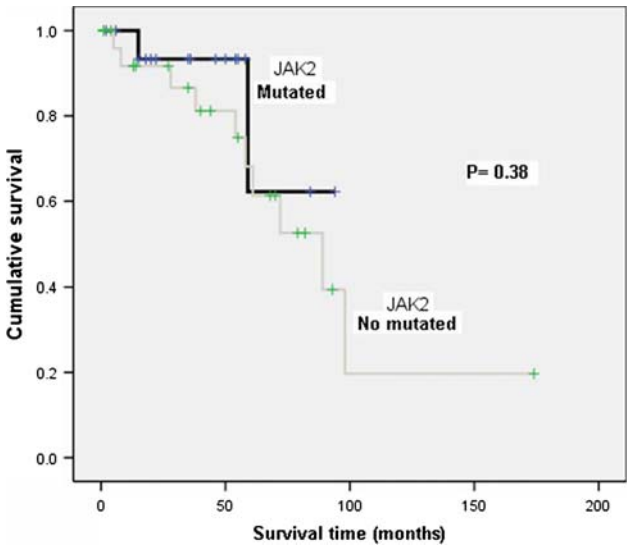

Fig. 3 Kaplan–Meier survival curves of patients with RARS-T according to the presence or absence of JAK2 V617F mutation

4 Discussion

According to the WHO classification of the myeloid neoplasms, diagnosis of RARS-MT requires more than 15% of ringed sideroblasts in bone marrow aspirate and a platelet count above 600 × 10^9/L in peripheral blood. This "cut-off" platelet count is considered as arbitrary by some authors and the term "refractory anemia with ringed sideroblasts associated with thrombocytosis" (RARS-T) is preferred [4]. Nevertheless, when we compared in our study both the groups of patients, RARS-MT and RARS-nMT, as previously defined, some significant differences were found. Clinicopathological features of patients with RARS-MT were nearer to those of chronic myeloproliferative diseases, such as essential thrombocythemia and primary myelofibrosis (casual finding as a more frequent form of presentation, higher WBC count, higher incidence of splenomegaly, higher frequency of marrow megakaryocytic hyperplasia and reticulin fibrosis in bone marrow trephines, and presence of JAK2 V617F mutation in a higher percentage of cases), whereas patients with RARS-nMT had initial manifestations and clinical evolution nearer to myelodysplastic syndromes (presentation as anemic syndrome, lower hemoglobin level, higher MCV,

and less incidence of JAK2 V617F mutation). Thus, platelet count of 600 $\times$ 10^9/L seems not to be so arbitrary, and in our series could separate patients with myeloproliferative disease from those with a myelodysplastic behavior.

Even though an underlying myeloproliferative disorder constitutes a risk factor for thrombosis (mostly arterial events) [5], the incidence of thrombotic complications in our series is rather low, even in patients carrying the JAK2 mutation. Leukocytosis, but not thrombocytosis, has been identified as a potential risk factor for thrombosis in polycythemia vera and essential thrombocythemia; a high WBC count was rare in the group of patients with RARS-T that we have studied (only 9 of the 76 patients had a WBC above 11 $\times$ 10^9/L at diagnosis, and none of them exceeded 18 $\times$ 10^9/L).

Concerning morphological results from peripheral blood and bone marrow aspirate smears, we observed that patients with RARS-MT and RARS-nMT have both dysplastic and proliferative features at the time of initial presentation. The presence of basophilic stippling and abnormal hemoglobin distribution in erythrocytes (peripheral blood) and erythroblasts (bone marrow) was a constant finding. This indicates a sideroacrestic phenomenon that can be verified by an iron stain as ringed sideroblasts. We could not correlate the morphological findings with either the level of platelets or the mutational status of JAK2.

Although some authors do not support the descent of the platelet count from 600 $\times$ 10^9/L to 500 $\times$ 10^9/L to establish the diagnosis of RARS-MT [6], the existence of three cases carrying the JAK2 mutation in our group of RARS-nMT, forced us to revaluate the patients. These three patients had a platelet count between 500 $\times$ 10^9/L and 600 $\times$ 10^9/L. As result of this new comparison, we could find that only JAK2 mutational status and megakaryocytic hyperplasia degree at bone marrow biopsy were clearly different. With this new "cut-off" other differences that previously seem to separate more myeloproliferative (RARS-MT) from more myelodysplastic (RARS-nMT) "behaviors" between the patients disappeared.

We have to consider that in 2001, when the WHO classification of myeloid neoplasms was published, the JAK2 mutation and its importance in the study of myeloproliferative disorders was unknown [7]. Now there is increasing evidence concerning the crucial physiopathological role of JAK2 V617F mutation in a high proportion of patients with chronic myeloproliferative disease. In fact, more than 90% of patients affected by polycythemia vera and approximately 50% of those with essential thrombocythemia or primary myelofibrosis are carriers of the somatic JAK2 V617F mutation in their granulocytes [8]. These findings have led some authors to reconsider the WHO classification of Philadelphia-negative chronic myeloproliferative diseases [9–12]. Although much more uncommon, this mutation also may be detected in other atypical myeloproliferative disorders, and very occasionally in myelodysplastic syndromes [13–15]. Some authors have detected the presence of this mutation in myelodysplastic syndrome-derived leukemia of megakaryoblastic nature [16]. Finally, JAK2 V617F mutation has not been described in association to secondary or reactive leukocytosis or thrombocytosis, or in lymphoproliferative diseases. In consequence, the analysis of the JAK2 mutational status should be considered as a myeloid-specific clonality test, and is very useful in the evaluation of Philadelphia-negative chronic myeloproliferative diseases.

Taking into account our results, combination of platelet count and JAK2 mutational status could help to separate clinically-different patients with RARS-T. Derived from the retrospective design of our study, determination of JAK2 V617F mutation was performed in 47 patients (62% of the collected cases). Globally, the mutation was present in 17/47 cases (36%), but the incidence significantly increased in RARS-MT group (14/23, 61%), when compared to RARS-nMT (3/24, 12.5%). Some authors have reported a higher incidence of JAK2 mutation in patients with RARS-T [15, 17–24]. The frequency of this mutation is similar to that observed in our series (61%), which is the largest reported. Together with higher platelet count, we found that patients with JAK2 mutation presented with higher leukocyte count, higher frequency of splenomegaly, and higher frequency of bone marrow fibrosis. On the other hand, patients without the mutation showed lower hemoglobin concentration and higher MCV. Hydroxyurea used as cytoreduction treatment was clearly superior in patients carrying this mutation, while packed red cell transfusion requirement was superior in the group of patients without JAK2 mutation.

Recently, a German group has reported that RARS-T displays distinct clinicopathological and molecular features which are different from those of classical essential thrombocythemia and primary myelofibrosis [24]. Our results, derived from the retrospective study of 76 patients, support the concept that RARS-T could include two groups, RARS-MT and RARS-nMT, the former presenting as chronic myeloproliferative disease associated to JAK2 V617F mutation and the latter with clinicobiological features of myelodysplastic syndrome, usually not associated to JAK2 mutation. Nevertheless, the absence of significant differences between RARS-MT and RARS-nMT groups and between JAK2-mutated and JAK2-non mutated groups, in terms of overall median survival, constitutes an interesting finding which needs to be further investigated. In agreement with other recent report [25], we think that diagnostic criteria for RARS-T need to be better defined, and as suggested by the authors, further studies which take into account other pathophysiological aspects of this entity are required.

5 Conclusions

Based on the above-mentioned findings in a large number of patients with RARS-T, a platelet count of 600 × 10^9/L seems to separate two groups: RARS with not-marked thrombocytosis (400–600 × 10^9/L), with biological features nearer to "classical" RARS and clinical behavior of myelodysplastic syndrome, and RARS associated with marked thrombocytosis (>600 × 10^9/L), that join characteristics of patients with chronic myeloproliferative disease. The establishment of a platelet count of 500 × 10^9/L as "cut-off" point instead of 600 × 10^9/L, allows to bring together all the RARS-T patients carrying the JAK2 mutation in the same group (that above 500 × 10^9/L), but clinical and analytical differences tend to disappear between both the groups. Morphological findings of this study provided evidence that RARS includes a wide spectrum of conditions ranging from myelodysplastic syndromes to myeloproliferative disorders (essential thrombocythemia, prefibrotic primary myelofibrosis). In our opinion, although a "gray" zone persists between 500 × 10^9/L and 600 × 10^9/L, the combination of platelet count and JAK2 V617F mutation status may help to separate RARS-T cases with more myeloproliferative features (JAK2 mutated) from those with myelodysplastic behaviour (JAK2 not-mutated).

Aknowledgments This work has been partially supported by a FIS grant (PI030345) and a FEHH grant (*Fundación Española de Hematología y Hemoterapia*). Spanish Hospitals, Cities And Doctors′ Name: H. del Mar, Barcelona (L Florensa, L Arenillas, B Bellosillo, S Woessner); H. Universitari de Bellvitge, Hospitalet, Barcelona (A Domingo, E Alonso); H. Clinic i Provincial, Barcelona (M Rozman, G Gutiérrez); H. Central de Asturias (E Luño, C Sanzo); H. de Cruces, Baracaldo, Vizcaya (MA Piñán, G Letamendi); H. La Fe, Valencia (ML Perez-Sirvent, J Cervera); H. de León, León (M Barbón); H. de Getafe, Madrid (L García); H. Doctor Negrín, Las Palmas (A Lemes, T Molero); H. Marqués de Valdecilla, Santander (L Yáñez); H. Sierrallana, Torrelavega, Cantabria (JI Olalla, MJ Muruzabal); H. Germans, Trias y Pujol, Badalona, Barcelona (F Millá, JT Navarro); H. Txagorritxu, Vitoria (M Ardanaz); H. de Zumárraga, Guipúzcoa (A Elosegui, T Hernandez-Santamaría); H. Clínico San Carlos, Madrid (A Villegas, M Mateo); H. de Laredo, Cantabria (MA Cortés, ML González-Ponte); H. Son Dureta, Palma de Mallorca (MA Durán); H. Vall d′Hebrón (T Vallespí); and H. Universitario de Canarias, La Laguna (JM Raya, T Martín, L Morabito, L Hernández-Nieto).

References

1. Jaffe ES, Harris NL, Stein H, Vardiman JW. WHO classification. Tumours of haematopoietic and lymphoid tissues. Lyon: IARC press; 2001.
2. Raya JM, Florensa L, Domingo A, Arenillas L, Alonso E, Letamendi G, et al. Refractory anaemia with ringed sideroblasts associated with thrombocytosis: results from a Spanish multicentric study. In: 12th European Haematology Association (EHA) Congress, Vienna, abstract 1137; 2007.
3. Levine RL, Belisle C, Wadleigh M, Zahrieh D, Lee S, Chagnon P, et al. X-inactivation-based clonality analysis and quantitative JAK2V617F assessment reveal a strong association between clonality and JAK2V617F in PV but not in ET/MMM, and identifies a subset of JAK2V617F-negative ET and MMM patients with clonal hematopoiesis. Blood. 2006;107:4139–41. doi:10.1182/blood-2005-09-3900.
4. Shaw GR. Ringed sideroblasts wit thrombocytosis: an uncommon mixed myelodysplastic/myeloproliferative disease of older adults. Br J Haematol. 2005;131:180–4.
5. Tefferi A, Elliott M. Thrombosis in myeloproliferative disorders: prevalence, prognostic factors, and the role of leukocytes and JAK2V617F. Semin Thromb Hemost. 2007;33:313–20. doi:10.1055/s-2007-976165.
6. Bain B. Ringed sideroblasts wit thrombocytosis: an uncommon mixed myelodysplastic/myeloproliferative disease of older adults. Br J Haematol. 2006;134:340. doi:10.1111/j.1365-2141.2006.06172.x.
7. Tefferi A, Gilliland DG. JAK2 in myeloproliferative disorders is not just another kinase. Cell Cycle. 2005;4:1053–6.
8. Tefferi A, Pardanani A. Mutation screening for JAK2V617F: when to order the test and how to interpret the results. Leuk Res. 2006;30:739–44. doi:10.1016/j.leukres.2006.01.004.
9. Tefferi A, Gilliland DG. The JAK2V617F tyrosine kinase mutation in myeloproliferative disorders: status report and immediate implications for disease classification and diagnosis. Mayo Clin Proc. 2005;80:947–58.
10. Tefferi A. Classification, diagnosis and management of myeloproliferative disorders in the JAK2V617F era. Hematology (Am Soc Hematol Educ Program). 2006; 240–5. doi:10.1182/asheducation-2006.1.240.
11. Michiels JJ, De Raeve H, Berneman Z, Van Bockstaele D, Hebeda K, Lam K, et al. The 2001 World Health Organization and updated European clinical and pathological criteria for the diagnosis, classification, and staging of the Philadelphia chromosome-negative chronic myeloproliferative disorders. Semin Thromb Hemost. 2006;32:307–40. doi:10.1055/s-2006-942754.
12. Michiels JJ, Bernema Z, Van Bockstaele D, De Raeve H, Schroyens W. Current diagnostic criteria for the chronic myeloproliferative disorders (MPD) essential thrombocythemia (ET), polycythemia vera (PV) and chronic idiopathic myelofibrosis (CIMF). Pathol Biol (Paris). 2007;55:92–104. doi:10.1016/j.patbio.2006.06.002.
13. Steensma DP, Dewald GW, Lasho TL, Powell HL, McClure RF, Levine RL, et al. The JAK2 V617F activating tyrosine kinase mutation is an infrequent event in both "atypical" myeloproliferative disorders and myelodysplastic syndromes. Blood. 2005;106:1207–9. doi:10.1182/blood-2005-03-1183.
14. Ohyashiki K, Aota Y, Akahane D, Gotoh A, Miyazawa K, Kimura Y, et al. The JAK2 V617F tyrosine kinase mutation in myelodysplastic syndromes (MDS) developing myelofibrosis indicates the myeloproliferative nature in a subset of MDS patients. Leukemia. 2005;19:2359–60. doi:10.1038/sj.leu.2403989.
15. Wang SA, Hasserjian RP, Loew JM, Sechman EV, Jones D, Hao S, et al. Refractory anemia with ringed sideroblasts associated with marked thrombocytosis harbors JAK2 mutation and shows overlapping mieloproliferative and myelodysplastic features. Leukemia. 2006;20:1641–4. doi:10.1038/sj.leu.2404316.
16. Nishii K, Nanbu R, Lorenzo VF, Monma F, Kato K, Ryuu H, et al. Expression of the JAK2 V617F mutation is not found in de novo AML and MDS but is detected in MDS-derived leukemia of megakaryoblastic nature. Leukemia. 2007;21:1337–8. doi:10.1038/sj.leu.2404626.
17. Szpurza H, Tiu R, Murugesan G, Aboudola S, His ED, Theil KS, et al. Refractory anemia with ringed sideroblasts associated with

marked thrombocytosis (RARS-T), another myeloproliferative condition characterized by JAK2 V617F mutation. Blood. 2006;108:2173–81. doi:10.1182/blood-2006-02-005751.
18. Remacha AF, Nomdedéu JF, Puget G, Estivill C, Sarda P, Canals C, et al. Occurrence of the JAK2 V617F mutation in the WHO provisional entity: myelodysplastic/myeloproliferative disease, unclassifiable-refractory anemia with ringed sideroblasts associated with marked thrombocytosis. Haematologica. 2006;91:719–20.
19. Boissinot M, Garand R, Hamidou M, Hermouet S. The JAK2–V617F mutation and essential thrombocythemia features in a subset of patients with refractory anemia with ring sideroblasts (RARS). Blood. 2006;108:1781–2. doi:10.1182/blood-2006-03-008227.
20. Renneville A, Quesnel B, Charpentier A, Terriou L, Crinquette A, Laï JL, et al. High occurrence of JAK2 V617F mutation in refractory anemia with ringed sideroblasts associated with marked thrombocytosis. Leukemia. 2006;20:2067–70. doi:10.1038/sj.leu.2404405.
21. Steensma DP, Caudill JS, Pardanani A, McClure RF, Lasho TL, Tefferi A. MPL W515 and JAK2 V617 mutation analysis in patients with refractory anemia with ringed sideroblasts and an elevated platelet count. Haematologica. 2006;91:ECR57.
22. Ceesay MM, Lea NC, Ingram W, Westwood NB, Gaken J, Mohamedali A, et al. The JAK2 V617F mutation is rare in RARS but common in RARS-T. Leukemia. 2006;20:2060–1. doi:10.1038/sj.leu.2404373.
23. Gattermann N, Billiet J, Kronenwett R, Zipperer E, Germing U, Nollet F, et al. High frequency of the JAK2 V617F mutation in patients with thrombocytosis (platelet count >600 $\times$ 10^9/L) and ringed sideroblasts more than 15% considered as MDS/MPD, unclassifiable. Blood. 2007;109:1334–5. doi:10.1182/blood-2006-05-022491.
24. Schmitt-Graeff AH, Teo SS, Olschewski M, Schaub F, Haxelmans S, Kirn A, et al. JAK2 V617F mutation status identifies subtypes of refractory anemia with ringed sideroblasts associated with marked thrombocytosis. Haematologica. 2008;93:34–40. doi:10.3324/haematol.11581.
25. Malcovati L, Cazzola M. Myelodysplastic/myeloproliferative disorders. Haematologica. 2008;93:4–6. doi:10.3324/haematol.11374.