MYELOID NEOPLASIA

Impact of calreticulin mutations on clinical and hematological phenotype and outcome in essential thrombocythemia

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Key Points

- *CALR* mutations occur in half of *JAK2* and *MPL* wt patients with ET and associate with some distinctive phenotypic traits.
- Patients with ET harboring *CALR* mutations are at significantly lower risk of thrombosis compared with *JAK2*- and *MPL*-mutated patients.

Mutations in the calreticulin (*CALR*) gene were recently discovered in patients with essential thrombocythemia (ET) lacking the *JAK2*V617F and *MPL*W515 mutations, but no information is available on the clinical correlates. In this series, *CALR* mutations were found in 15.5% of 576 World Health Organization–defined ET patients, accounting for 48.9% of *JAK2* and *MPL* wild-type (wt) patients. *CALR*-mutated patients were preferentially male and showed higher platelet count and lower hemoglobin and leukocyte count compared with *JAK2*- and *MPL*-mutated patients. Patients carrying the *CALR* mutation had a lower risk of thrombosis than *JAK2*- and *MPL*-mutated patients; of interest, their risk was superimposable to patients who were wt for the above mutations. *CALR* mutation had no impact on survival or transformation to post-ET myelofibrosis. Genotyping for *CALR* mutations represents a novel useful tool for establishing a clonal myeloproliferative disorder in *JAK2* and *MPL* wt patients with thrombocytosis and may have prognostic and therapeutic relevance. (*Blood*. 2014;123(10):1552-1555)

Introduction

Unlike polycythemia vera (PV), where virtually all the patients harbor *JAK2* mutations (*JAK2*V617F in >95% and *JAK2* exon 12 mutations in ~2-4%), only 50% to 60% of essential thrombocythemia (ET)[1-3] and primary myelofibrosis (PMF)[4-6] patients have *JAK2*V617F mutation (*JAK2*$^+$). An additional 3% to 5% of ET and PMF subjects present *MPL* mutations at codon 515 (*MPL*$^+$).[7-9] *JAK2* and *MPL* mutations represent major diagnostic criteria in the 2008 World Health Organization (WHO) classification of chronic myeloproliferative neoplasms (MPN).[10,11] All these mutations result in the abnormal activation of the Janus kinase/signal transducer and activator of transcription signaling pathway that represents a hallmark of MPN and a target for therapy.[12] Recently, mutations at exon 9 of *CALR*, the gene encoding calreticulin, an endoplasmic reticulum Ca^{2+}-binding chaperone, were discovered in 50% to 70% of patients with ET and PMF (*CALR*$^+$) who were wild type (wt) for *JAK2* and *MPL*.[13,14] The mechanisms by which *CALR* mutations produce a myeloproliferative phenotype are unknown.

The aim of this study was to describe the prevalence, characteristics, and clinical and laboratory features associated with *CALR* mutations in a large population of patients with WHO-defined ET.

Study design

The study involved 576 patients with a diagnosis of ET fulfilling the 2008 WHO criteria who were followed at the Hematology Department, University of Florence. They had a stored sample of granulocyte DNA collected at diagnosis or within 3 years. Patients had provided an informed written consent in accordance with the Declaration of Helsinki for the use of DNA for investigational purposes. The Ethical Committee was that of the Azienda Ospedaliero-Universitaria Careggi in Florence. The *JAK2*V617F and *MPL*W515L/K mutations were assessed by real-time quantitative polymerase chain reaction[15,16] and also by high-resolution melting analysis followed by bidirectional Sanger sequencing for *MPL*.[17] Mutations in exon 9 of *CALR* were assessed by bidirectional sequencing.[13]

Patient characteristics reported in Table 1 were obtained at diagnosis. Splenomegaly was defined as a palpable organ below the left costal margin. Major thrombosis and bleeding, at diagnosis and/or in the 2 preceding years and/or anytime during follow-up, were recorded when objectively documented[18] and according to standard definitions.[19] Microvessel symptoms consisted of erythromelalgia and recurrent episodes of otherwise unexplained blurred vision, tinnitus, paresthesia, and headache. Constitutional symptoms included fever, unintentional weight loss, and night sweats. Pruritus was recorded when described as a diffuse, recurrent itching exacerbated by water contact. Evolution to post-ET myelofibrosis (PET-MF) and acute leukemia (AL) was diagnosed following the International Working Group for Myeloproliferative

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Table 1. Laboratory and clinical characteristics of *CALR* mutant patients compared with *JAK2V617F* or *MPL*W515 mutant patients and patients who were wt for the 3 mutations

					P value		
	CALR$^+$	*JAK2* V617F$^+$	*MPL* W515$^+$	*CALR, JAK2, MPL* wt	*CALR*$^+$ vs *JAK2* V617F$^+$	*CALR*$^+$ vs *MPL* W515$^+$	*CALR*$^+$ vs *CALR, JAK2, MPL* wt
Number of patients (%)	89 (15.5)	369 (64.1)	25 (4.3)	93 (16.1)	—	—	—
Male, no. (%)	53 (59.5)	117 (31.7)	6 (24.0)	18 (19.4)	**<0.0001**	**0.002**	**<0.0001**
Age, years	54.7 (13-88)	61 (15-93)	54 (22.89)	53 (15-87)	**0.04**	0.997	0.519
Leukocyte count ($\times 10^9$/L)	8.1 (3.5-26.0)	8.9 (4.2-35.0)	8.4 (4.5-16.6)	8.3 (4-16.8)	**0.001**	0.834	0.367
Hemoglobin (g/L)	138 (106-173)	145 (102-173)	136 (110-160)	136 (106-164)	**<0.0001**	0.315	0.380
Hematocrit (%)	41.2 (35.9-49.4)	43.8 (31.4-53.6)	41.2 (32.8-50)	41.0 (31.3-51.5)	**<0.0001**	0.887	0.893
Platelet count ($\times 10^9$/L)	866 (504-2348)	726 (455-1881)	898 (607-2000)	697 (482-1659)	**<0.0001**	0.385	**<0.0001**
Lactate dehydrogenase (U/L)	320 (142-725)	288 (102-1178)	365 (254-570)	268 (137-554)	0.307	0.665	**<0.01**
Splenomegaly, no. (%)	24 (27.0)	91 (24.7)	9 (36.0)	9 (9.7)	0.661	0.416	**0.004**
Pruritus, no. (%)	5 (5.6)	32 (8.7)	1 (4.0)	9 (9.7)	0.260	0.847	0.228
Constitutional symptoms, no. (%)	1 (1.1)	19 (5.1)	1 (1.2)	6 (6.5)	0.120	0.577	0.078
Major thrombosis, no. (%)	12 (13.5)	111 (30.1)	10 (40.0)	15 (16.1)	**0.011**	**0.012**	0.894
Microvessel symptoms, no. (%)	22 (24.7)	101 (27.4)	14 (56.0)	20 (21.5)	0.604	**0.003**	0.674
Major hemorrhage, no. (%)	4 (4.5)	17 (4.6)	4 (16.0)	3 (3.3)	0.906	0.067	0.587
Progression to PET-MF, no. (%)	4 (4.5)	12 (3.3)	2 (8.0)	1 (1.1)	0.458	0.563	0.128
Progression to PV, no. (%)	0	5 (1.4)	0	0	0.294	—	—
Progression to AL, no. (%)	0	2 (0.5)	1 (4.0)	1 (1.1)	0.507	0.071	0.349
Deceased (n = 70) (%)	10 (11.2)	49 (13.3)	4 (16.0)	7 (7.5)	0.598	0.515	0.414
Cytoreductive therapy, no. (%)	50 (62.5)	220 (60.8)	19 (76.0)	40 (46.0)	0.440	0.160	**0.023**

Hematologic and clinical information was collected at diagnosis; information regarding major thrombosis and hemorrhage included events at diagnosis, in the 2 preceding years, and during follow-up. Cytoreduction means that the patient received cytoreductive drugs (in >90% of cases, this was hydroxyurea) during the course of the disease at the physician's discretion, based on conventional criteria. Unless otherwise indicated, values are reported as median (range). Statistically significant differences are shown in bold.

Neoplasms Research and Treatment and WHO criteria, respectively.[11,20] Patients were treated according to current recommendations[21]; cytoreduction was hydroxyurea in >90% of high-risk patients.

Statistical analysis was performed with SPSS software. Patient characteristics were compared with the use of the χ^2 test or Fisher's exact test for categorical variables and the *t*-test or nonparametric test for continuous variables. The significance level was $P < .05$ in 2-sided tests. Survival estimates were obtained with the Kaplan-Meier method; the hazard ratio was determined using a Cox proportional hazards model.

Results and discussion

In the entire patient series, the median age was 58.1 years (range, 13-93 years); 194 subjects (33.7%) were male. Median follow-up was 71.9 months (range, 2-257 months); 70 patients (12.1%) died a median of 53.9 months (range, 2-249 months) after diagnosis. Nineteen patients (3.3%) progressed to PET-MF after a median of 122 months (range, 19-248 months); transformation to PV was documented in 5 cases (0.9%), and 4 patients (0.7%) evolved to AL after a median of 117.7 months (range, 56-250 months). We found 89 patients (15.5% of total) harboring exon 9 *CALR* mutations. *CALR* mutations were represented by insertions and deletions, as previously reported.[13,14] Deletions (60.7%) occurred more frequently than insertions (39.3%); the most common deletion was del367fs46 (37.0%), and ins385fs47 (71.4%) was the most common among insertions. *JAK2*V617F and *MPL*W515 mutations occurred in 64.1% (n = 369) and 4.3% (n = 25) of patients. *CALR*$^+$ patients accounted for 48.9% of *JAK2* and *MPL* wt patients (n = 182); 93 patients (16.1% of total) were wt for the 3 mutations considered.

We compared hematological and clinical characteristics of the patients who were categorized according to their *JAK2*V617F, *MPL*W515, and *CALR* genotype (Table 1). *CALR*$^+$ patients were younger than *JAK2*$^+$ and no different from *MPL*$^+$ and wt; a striking male predominance was found among *CALR*$^+$ (59.5%) compared with *JAK2*$^+$ (31.7%; $P < .001$), *MPL*$^+$ (24.0%, $P = .002$), and wt (19.4%; $P < .001$) patients. Influence of gender on *JAK2*V617F allele burden,[22] disease class, and vascular complications[23] is well documented, and current data add to the understanding of the role of host variations for the expression of the MPN phenotype.[24]

The leukocyte count, hemoglobin, and hematocrit level were lower in *CALR*$^+$ compared with *JAK2*$^+$ ($P = .001$, $P < .0001$, and $P < .0001$, respectively) and were similar to *MPL*$^+$ and wt patients; on the other hand, the platelet count was higher in *CALR*$^+$ than in *JAK2*$^+$ and wt patients ($P < .0001$ for both), but comparable to *MPL*$^+$ patients who also differed significantly from *JAK2*$^+$ ($P < .001$). Lactate dehydrogenase level was lower in wt compared with patients with any mutation ($P < .001$). Constitutional symptoms and pruritus were similarly represented in the different groups; a palpable spleen was less common in wt patients compared with those harboring any mutation ($P < .01$). The proportion of *CALR*$^+$ patients who received cytoreduction was similar to *JAK2*$^+$ and *MPL*$^+$ and lower than wt patients (Table 1). Overall, these findings indicate that *CALR*$^+$ patients, similar to *MPL*$^+$ patients, present a phenotype associated with preferential expansion of the megakaryocytic lineage compared with favored erythropoiesis in *JAK2*$^+$ patients.

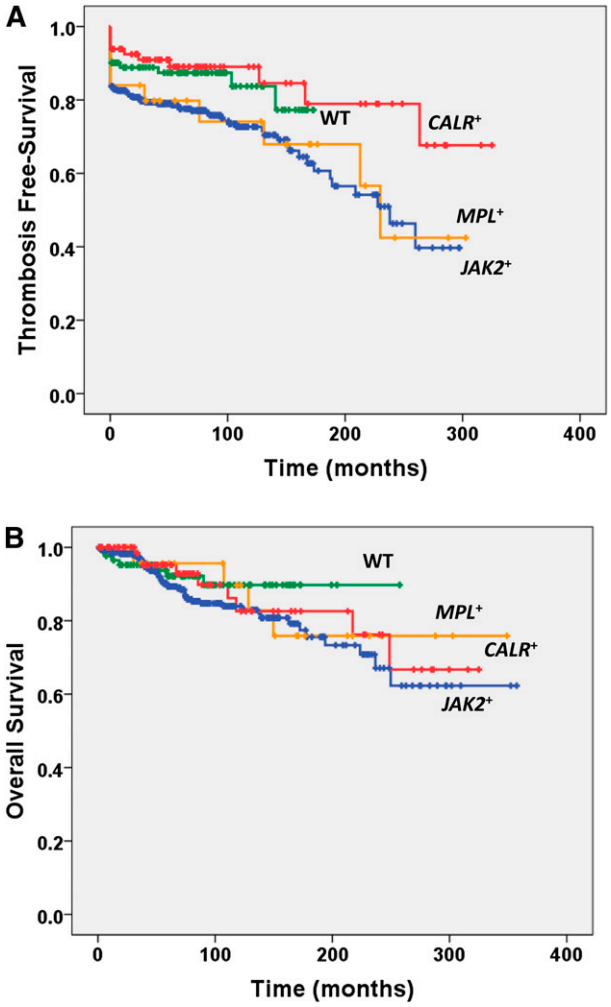

Figure 1. Overall Survival and Thrombosis-free survival according to mutational status. Kaplan-Meier estimate of (A) thrombosis-free survival and (B) overall survival in patients who were categorized according to their mutational status (*JAK2V617*$^+$, *MPLW515*$^+$, *CALR*$^+$, or wt for the above mutations).

Major cardiovascular events occurred in 30.1%, 40.0%, 13.5%, and 16.1% of the *JAK2*$^+$, *MPL*$^+$, *CALR*$^+$, and wt patients, respectively; the difference was statistically significant when comparing *CALR*$^+$ vs *JAK2*$^+$ and *MPL*$^+$ patients ($P = .01$ for both). On the other hand, microvessel symptoms were more represented among *MPL*$^+$ patients ($P < .01$ compared with the other groups). The thrombosis-free survival was significantly longer in *CALR*$^+$ and wt patients compared with *JAK2*$^+$ and *MPL*$^+$ ($P = .008$; Figure 1A). The cumulative incidence of thrombosis at 10 years was 5.12% (95% confidence interval [CI], 1.6-15.2) in *CALR*$^+$, 14.54% (95% CI, 10.0-20.8) in *JAK2*$^+$, 19.46% (95% CI, 7.6-44.6) in *MPL*$^+$, and 8.17% (95% CI, 2.7-23.3) in wt patients. Taking wt patients as the reference population, the HR for thrombosis was 0.74 (95% CI, 0.33-1.00) for *CALR*$^+$, 1.78 (95% CI, 1.06-3.18) for *JAK2*$^+$, and 1.65 (95% CI, 1.7-3.92) for *MPL*$^+$ patients. There was a trend toward more frequent hemorrhages in *MPL*$^+$ compared with all other patients. The median survival was not reached in any group, and Kaplan-Meier estimates of survival did not show significant differences (Figure 1B). *CALR*$^+$ patients were preferentially distributed in the lower-risk category of the thrombosis score, the International Prognostic Score in Essential Thrombocythemia (IPSET), and the IPSET-thrombosis score compared with *JAK2*$^+$ (and *MPL*$^+$ for IPSET-thrombosis) (supplemental Table 1 available on the *Blood* website). Overall, these data indicate that *CALR*$^+$ patients are less prone to thrombotic events compared with *JAK2*$^+$ and *MPL*$^+$; of note, their risk was similar to patients lacking any mutations.

Transformation to PET-MF occurred in 19 patients; the hazard ratio for PET-MF was similarly increased in *CALR*$^+$ (2.36; 95% CI, 0.26-21.8), *JAK2*$^+$ (2.21; 95% CI, 0.28-17.8), and *MPL*$^+$ (2.50; 95% CI, 0.22-28.5) patients compared with wt, although, possibly due to a small number of events, this did not reach the significance level. Noteworthy, all 5 cases of transformation to PV occurred in the *JAK2*$^+$ patients.

With the limitations imposed by its observational nature, which precludes any causal relationships inferences, results of the current study identified meaningful associations between the presence of *CALR* mutations and the phenotype of patients with ET. The findings that *CALR*-mutated patients are at lower risk of vascular events may have implications for risk stratification and management. Finally, our study underscores the importance of *CALR* genotyping for an accurate diagnosis of patients with thrombocytosis who lack the *JAK2*V617F and *MPL*W515 mutations.

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Authorship

Contributions: P.G. and A.M.V. designed the study, analyzed the data, and wrote the manuscript; G.R. and C.M. performed molecular analysis and analyzed raw sequencing data; A. Pacilli, A. Pancrazzi, and T.F. contributed to molecular analysis; P.G., L.P., A.B., and A.M.V. contributed samples and clinical information; and all authors read the final version of the manuscript and agreed on its content. For a description of the AGIMM project and list of investigators, see www.progettoagimm.it.

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