CALR-mutated patients with low allele burden represent a specific subtype of essential thrombocythemia: A study on behalf of FIM and GBMHM.

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CALR-mutated patients with low allele burden represent a specific subtype of essential thrombocytopenia: A study on behalf of FIM and GBMHM

To the Editor:

BCR-ABL1-negative myeloproliferative neoplasms (MPNs) are clonal disorders characterized by an overproduction of myeloid mature cells, including essential thrombocytopenia (ET), primary myelofibrosis (PMF), and polycythemia vera (PV). Somatic gain-of-function mutations in one of the three driver genes (JAK2, CALR or MPL) are the causative events leading to the constitutive activation of the JAK/STAT pathway and are found in almost 80% of MPN cases. The quantification of the JAK2V617F allele burden in blood leukocytes, reflecting the size of the mutant clone, is highly variable from 1% to 100% in MPN patients and correlates with an increased risk of thrombosis and myelofibrotic evolution in PV and ET patients with an allele burden >50%. Some MPN patients display a low JAK2V617F allele burden in blood leukocytes (i.e. ≤20%). A recent study demonstrated that this is caused by a bias in clonal hematopoiesis with a late expansion of the mutated clone restricted to the erythroid and/or megakaryocytic lineages. Conversely, the allele burden of CALR mutations appeared to be less variable, with values around 40%–50% associated with clonal dominance in hematopoietic stem cells and progenitors compartments. In the literature, CALR-mutated MPNs with a low allele burden appear to be present in a small number of patients, but scarce data are available concerning the profile of these patients.

We aimed to characterize CALR-mutated MPN with a low allele burden (i.e. <20%) by screening our local cohort and subsequently 11 French hospitals for patients with low allele burden. This study was registered by the French authority CNIL (Commission nationale de l’informatique et des libertés, French Data Protection Authority, authorization ar22-0016v0) and a non-opposition of patients was obtained. CALR allele burden quantifications were performed at the time of diagnosis and prior to any cytoreductive treatment (details in Data S1). In our local cohort, a low allele burden was encountered in 15.4% of CALR-mutated MPN (22/143) (Figure S1A), and a global frequency of 10.4% in the multicentric screening involving a total of 1479 CALR-mutated MPNs was found (Figure S1B). Among low allele burden patients, the diagnostic of ET was found in the vast majority of cases (127 of 135 patients, 94%).

We therefore aimed to compare the 127 low allele burden ET with a multicentric control cohort of 207 CALR-mutated ET with an allele burden ≥20% at the time of diagnosis (details in Data S1). All comparisons are shown in Table S1. The median and range of CALR allele burden were of 14% [0.4–19.8] and 38% [20.3–64.6] in the low and high allele burden groups, respectively (Figure S2). The majority of patients (54%) with a low allele burden had an allele burden between 5% and 15%, whereas 4.5% of patients had an allele burden below 5%. The sex ratio was reverted, with a female predominance in low allele burden patients compared with a male predominance in patients with CALR allele burden ≥20% (M/F: 0.63 vs. 1.49, p = 0.0002). No difference was observed for age at the time of diagnosis (p = 0.473). ET patients with CALR low allele burden had lower platelets and neutrophils counts, and lower LDH levels than CALR-mutated ET patients with a CALR allele burden ≥20% (p = 0.0005, p = 0.0008 and p = 0.009, respectively; Figure S3). Among CALR-mutated ET, the subtype of CALR mutation influenced the phenotype with higher platelet counts in type 2/2-like patients. However, the phenotype observed in low allele burden CALR-mutated ET is not linked to the subtype of CALR mutation because the distribution of mutation subtypes was similar between the two groups, with a majority of type 1/1-like mutations (53% and 57% of patients for low and high allele burden, respectively).

In terms of prognosis, CALR low allele burden ET exhibited a better overall survival than ET patients with a CALR allele burden ≥20% (Figure 1A). Multivariable analysis confirmed the better overall survival of CALR low allele burden and showed that age >60 years and a previous history of thrombosis were also independently associated with a decreased overall survival (Figure 1B). No differences were found regarding hematological transformations (Figure 1C) and thrombosis-free survival, although CALR low allele burden ET patients may have a lower long-term risk of thrombosis (Figure 1D). Nevertheless, the follow-up period for this cohort is limited (with a median duration of 51 months for low allele burden), and a longer follow-up would provide more conclusions regarding the impact of CALR allele burden on hematological transformations and thrombosis. Compared with JAK2V617F driver mutation, CALR mutations are associated with a better prognostic in PMF, but no difference was found in ET in terms of overall survival, thrombotic events, or fibrotic transformation. Thus, CALR-mutated ET patients with a low allele burden seemed to represent a subtype of ET with a more favorable prognosis.

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We then aimed to study the evolution of CALR allele burden in low allele burden patients. Follow-up samples were available for 26 CALR low allele burden ET patients, providing a total of 80 CALR measurements with a mean follow-up of 6 years between diagnosis and the last CALR quantification. Fifteen of the 26 patients (58%) remained with a low CALR allele burden below 20%, while 11 patients (42%) had an increase of CALR allele burden during their follow-up (Figure 1E). Three of the 26 patients progressed to secondary myelofibrosis, and all of them had an increase of their allele burden. This increase was observed at the time of myelofibrotic transformation for
two patients and 40 months before for the third patient. An increase
of CALR allele burden could be related to an increase of the clone size
and/or to a transition to homozygosity by a loss of heterozygosity
(LOH) at chromosome 19. Such homozygosity is a rare event but asso-
ciated with disease progression. A previous study also reported the
prognostic impact of an increase of CALR allele burden in a cohort of
ET patients.

Sixteen of the 26 patients were treated by cytoreductive therapy
during follow-up: 6 by hydroxyurea, 7 by interferon-α, and 3 by anagrelide. Treatment by interferon-α was not associated with a decrease of CALR allele burden, as 4 out of 7 patients showed an increase during follow-up.

Finally, we aimed to investigate whether a clonal expansion restricted to a lineage exists in CALR MPN, similarly to what has been described in MPN with low allele burden JAK2 mutation. We quantified CALR mutations in platelets, granulocytes, monocytes, B cells, T cells, NK cells, hematopoietic stem cells (CD34+CD38-, HSC), and hematopoietic progenitor cells (CD34+CD38+, HPC) for 19 CALR-mutated patients, including 13 patients undergoing treatment (Table S2 and Figure S4A). Median time between diagnosis and cell sorting was 6 years [2.76;13.48] (Figure S4A). The median allele burden of CALR mutation in total leukocytes was of 38%, and 6 of the 19 patients had low allele burden (i.e., <20%). The allelic burden of CALR mutation was relatively stable among HSC, HPC, and myeloid lineages (Figure S4B). For some patients, we found a lower CALR allele burden in platelets. Among lymphoid lineages, NK cells had the higher level of CALR allele burden, followed by B cells and lastly T cells, which were mostly non-clonal. These results indicate that a skewed expansion of mutated hematopoiesis is not present in CALR-mutated MPN. Future research may help elucidate the mechanisms by which a small clone in all compartments contributes to the observed phenotype.

Overall, our results indicate that CALR-mutated ET patients with a low allele burden exhibit a milder phenotype compared with CALR-mutated ET patients with an allele burden ≥20%. A low allele burden of CALR mutation at the time of diagnosis also influences the disease course with a better overall survival independently of classical prognostic markers such as age and previous history of thrombosis. In this retrospective real-world study, the diagnoses of ET were determined locally by a multidisciplinary team; however, we cannot exclude the possibility that certain cases may actually represent pre-fibrotic forms. Future large-scale studies with long-term follow-up and systematic monitoring of allele burden are essential for a more comprehensive understanding of the dynamics of evolution of these patients.

AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST STATEMENT
All authors declare no competing financial interests related to this work.

DATA AVAILABILITY STATEMENT
Data available on request due to privacy/ethical restrictions (individual data cannot be shared without a new regulatory approval).


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