Antiplatelet therapy *versus* observation in low-risk essential thrombocythemia with a *CALR* mutation

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ABSTRACT

The role of antiplatelet therapy as primary prophylaxis of thrombosis in low-risk essential thrombocythemia has not been studied in randomized clinical trials. We assessed the benefit/risk of low-dose aspirin in 433 patients with low-risk essential thrombocythemia (271 with a *CALR* mutation, 162 with a *JAK2*^{V617F} mutation) who were on antiplatelet therapy or observation only. After a follow up of 2215 person-years free from cytoreduction, 25 thrombotic and 17 bleeding episodes were recorded. In *CALR*-mutated patients, antiplatelet therapy did not affect the risk of thrombosis but was associated with a higher incidence of bleeding (12.9 *versus* 1.8 episodes per 1000 patient-years, *P*=0.03). In *JAK2*^{V617F}-mutated patients, low-dose aspirin was associated with a reduced incidence of venous thrombosis with no effect on the risk of bleeding. Coexistence of *JAK2*^{V617F}-mutation and cardiovascular risk factors increased the risk of thrombosis, even after adjusting for treatment with low-dose aspirin (incidence rate ratio: 9.8; 95% confidence interval: 2.3-42.3; *P*=0.02). Time free from cytoreduction was significantly shorter in *CALR*-mutated patients with essential thrombocythemia than in *JAK2*^{V617F}-mutated ones (median time 5 years and 9.8 years, respectively; *P*=0.0002) and cytoreduction was usually necessary to control extreme thrombocytosis. In conclusion, in patients with low-risk, *CALR*-mutated essential thrombocythemia, low-dose aspirin does not reduce the risk of thrombosis and may increase the risk of bleeding.

Introduction

Essential thrombocythemia (ET) is a myeloproliferative neoplasm characterized by an increased risk of thrombosis and hemorrhage. Patients older than 60 years or with a previous history of thrombotic complications are considered at high risk of thrombosis and managed with cytoreduction and antiplatelet agents. In contrast, watchful waiting, with or without antiplatelet therapy, is the recommended approach for younger patients in the absence of a history of thrombosis.[1,2]

The role of antiplatelet therapy as primary prophylaxis of thrombosis in this group of low-risk ET patients has not been studied in randomized clinical trials. Recommendations for its use are based on the extrapolation to ET of results from the ECLAP study[3] conducted in patients with polycythemia vera, and some observational studies in ET.[4] Nevertheless, recent studies in low-risk ET have found that antiplatelet therapy is effective in the prevention of thrombosis only in patients with cardiovascular risk factors or those who bear the *JAK2*^{V617F} mutation, while it might increase the risk of bleeding in those with marked thrombocytosis.[5]

JAK2^{V617F} and *CALR* exon 9 mutations are the most frequent, mutually exclusive, genetic alterations in ET, being present in 60% and 20% of such patients, respectively.[6,7] Recent studies have suggested that *CALR*-positive ET can be considered a distinct clinical entity from *JAK2*^{V617F}-positive ET because of higher platelet counts and a lower incidence of thrombosis in the former.[8-12] This observation is especially relevant with regard to the use of antiplatelet therapy in *CALR*-mutated low-risk ET patients, in whom the increased risk of bleeding associated with higher platelet counts might offset the benefits of reducing the relatively low risk of thrombosis.

The aim of the present study was to assess the benefit-risk balance of using antiplatelet therapy in the primary prevention of thrombosis in patients with low-risk ET according to *CALR* and *JAK2*^{V617F} mutational status.

Methods

Study design

The medical records of patients diagnosed with *CALR*-mutated ET at several European institutions were reviewed. Patients were eligible for inclusion in the study if they were younger than 60 years at diagnosis of ET, had no prior history of thrombosis or major bleeding, and had not received cytoreductive therapy as the initial treatment for ET. An additional number of *JAK2*^{V617F}-mutated cases with similar clinical characteristics and follow-up were used as a control group for comparisons. Since time free from cytoreduction was longer in *JAK2*^{V617F}-mutated cases, patients with *CALR* and *JAK2*^{V617F} genotypes were included in a 2:1 proportion, with this allowing a similar time at risk in both groups of patients. The decision between starting treatment with low-dose aspirin or keeping the patient on careful observation without antiplatelet therapy was taken by the attending hematologist. The diagnosis of ET was established at the hospital of origin using the updated criteria of the World Health Organization.[13] Informed consent for the scientific use of the patients' clinico-hematologic data and biological samples was obtained as required by the local ethics committees.

In all patients the main clinico-hematologic data at presentation of ET were collected, including age, sex, cardiovascular risk factors, hemoglobin values, leukocyte and platelet counts, as well as *JAK2*^{V617F} and *CALR* mutational status. Further data included the therapeutic approach (starting and stopping dates, reasons for initiation and withdrawal), occurrence of thrombosis and bleeding, transformation into polycythemia vera, myelofibrosis or acute leukemia, and cause of death.

Outcomes

The primary outcome of the study was the occurrence of thrombosis, either arterial or venous. The safety outcome was major hemorrhage. Thrombosis was defined according to the International Classification of Diseases (ninth revision). Arterial thrombosis included stroke, transient ischemic attacks, retinal artery occlusion, coronary arterial disease, and peripheral arterial disease. Venous thrombosis included cerebral venous sinus thrombosis, deep-vein thrombosis, pulmonary thromboembolism, Budd-Chiari syndrome, and spleno-portal vein thrombosis. Minor occlusive events, such as erythromelalgia and superficial thrombophlebitis of the extremities, were not considered. Severe hemorrhage was defined as symptomatic bleeding in a critical organ or an overt hemorrhage requiring transfusion or associated with a decrease in hemoglobin ≥20 g/L without transfusion.

Statistical methods

For the purpose of the present study, the time at risk of thrombosis or hemorrhage was computed from the date of diagnosis of ET to the date of death, last follow-up, first thrombotic or bleeding event, start of cytoreductive therapy or initiation of oral anticoagulation, whichever occurred first. The incidence rate of thrombosis or bleeding while the patients were on low-dose aspirin or on observation alone was calculated as the number of events per 1000 patient-years of follow-up. The incidence rate method allows periods without low-dose aspirin to be accounted for in those patients who were started on this therapy after an initial period of careful observation or withdrew from it at some time during follow-up.

A multivariate analysis of factors influencing the incidence rate of thrombosis or bleeding was performed using Poisson regression. In Poisson regression, the exponentiated coefficients of covariates can be regarded as incidence rate ratios (IRR) and are comparable to the hazard ratios in Cox models. Variables analyzed for their independent association with the incidence rate of thrombosis or hemorrhage included age, sex, cardiovascular risk factors, hematologic values at diagnosis, presence of the *JAK2*^{V617F} or *CALR* mutation and whether the patient was on low-dose aspirin or not. Marked thrombocytosis was defined as a platelet count at diagnosis of >1000x10^9/L. Leukocytosis was defined as a leukocyte count at diagnosis of >10x10^9/L. Further models were fitted with interactions between low-dose aspirin and selected clinical or biological features of the patients, such as the presence of cardiovascular risk factors or the *JAK2*^{V617F} or *CALR* mutation. Since a physician's decision to initiate low-dose aspirin was not random but influenced by the patient's characteristics, a propensity score was calculated from the binary logistic regression of the initial clinical and laboratory features predicting antiplatelet therapy. The propensity score assigns every patient a probability of being in the low-dose aspirin group instead of the observation group, conditional on that patient's clinico-biological features at the diagnosis of ET, and it was forced into the Poisson models in order to control for confounding. All the statistical analyses were performed with Stata, version 11 (*www.stata.com*). The time-span splitting method[14] was used to calculate the incidence rates and fit the Poisson models.

Results

Characteristics of the patients and follow-up data

A total of 433 low-risk ET patients were included in the study, 271 were *CALR*-mutated and 162 carried the *JAK2*^{V617F} mutation. The type of mutation was known for 211 (78%) of the 271 *CALR*-positive patients (type 1 in 111; type 2 in 80; other types in 20). The main characteristics of the patients according to *JAK2*^{V617F} or *CALR* mutational status are shown in Table 1.

Cytoreductive therapy was started in 231 patients. The projected time from diagnosis to cytoreduction was significantly shorter in patients with *CALR*-mutated ET than in *JAK2*^{V617F}-mutated patients (median 5.0 years and 9.8 years, respectively; log-rank test *P*=0.002. Figure 1). Reasons for starting cytoreductive therapy are shown in Table 2. As can be seen, extreme thrombocytosis was the commonest reason for starting cytoreductive therapy in *CALR*-positive patients. First-line cytoreductive therapy included hydroxyurea (143 patients), anagrelide (66 patients), interferon (18 patients), and busulfan (4 patients). Some of these patients received second- and third-line cytoreductive therapy (*data not shown*).

Time at risk of thrombosis and bleeding, free of cytoreductive therapy, was 2215 person-years. Antiplatelet therapy with low-dose aspirin (81-100 mg/day) was started in 353 patients, either at diagnosis of ET or later during follow-up. Low-dose aspirin was withdrawn in 50 out of these 353 patients, either permanently (46 patients) or temporarily (4 patients). Taking into consideration the periods of time on or off antiplatelet therapy in every patient, time at risk of thrombosis or major bleeding was 1307 and 908 person-years for low-dose aspirin and observation only, respectively. With regard to mutational status, time at risk was 1192 and 1023 person-years for *CALR*-mutated and *JAK2*^{V617F}-mutated ET, respectively.

Incidence of thrombosis and major bleeding

A total of 25 thrombotic events (arterial or venous) were recorded over the 2215 person-years of follow-up time in which patients remained in the low-risk status free from cytoreductive therapy. Fourteen out of these 25 thrombotic events occurred while patients were receiving low-dose aspirin whereas 11 thromboses appeared while patients were on observation only, resulting in an incidence rate of 10.7 and 12.1 thrombotic events per 1000 person-years, respectively (Figure 2, *P*=0.7). *CALR*-positive patients had an incidence rate of thrombosis of 9.7 and 6.9 events per 1000 person-years while on antiplatelet therapy and careful observation, respectively (Figure 2, *P*=0.6). In the *JAK2*^{V617F}-mutated patients, the incidence rate of thrombosis was higher while they were on observation only without aspirin but the difference did not attain statistical significance (21.1 *versus* 11.6 events per 1000 person-years for observation and aspirin, respectively, *P*=0.3, Figure 2).

Fourteen arterial thrombotic events were recorded during the period at risk. The incidence rate of arterial thrombosis was similar for periods on and off antiplatelet therapy, both in the whole cohort and the subgroups of patients with *CALR* or *JAK2*^{V617F} mutations (Figure 3). Venous thrombosis was observed in 11 patients, four of them were on antiplatelet therapy and seven on careful observation (incidence rate: 3.1 and 7.7 episodes per 1000 person-years, respectively; *P*=0.1, Figure 4). In the *JAK2*^{V617F}-mutated patients, there was a significantly higher rate of venous thrombosis when patients were on careful observation than when they were on antiplatelet therapy (15 *versus* 2.9 events per 1000 person-years, respectively, *P*=0.045, Figure 4). In contrast, in the *CALR*-mutated patients, no significant association was found between low-dose aspirin and the incidence rate of venous thrombosis (Figure 4).

A multivariate model including age, sex, presence of cardiovascular risk factors leukocyte count at diagnosis, type of mutation (*CALR* or *JAK2*^{V617F}) and whether the patient was on or off antiplatelet therapy did not identify any risk factor for thrombosis (arterial and venous together). On interaction analyses, patients with both *JAK2*^{V617F} mutation

Table 1. Main clinico-hematologic characteristics at diagnosis of 433 patients with low-risk essential thrombocythemia.

	JAK2^{V617F} n=162	CALR n=271	*P*
Age, years*	42 (10-59)	42 (12-59)	0.5
Sex M/F, %	35/65	44/56	0.055
Cardiovascular risk factors, n (%)	79 (49)	74 (27)	0.0001
Microvascular symptoms, n (%)	38 (24)	66 (25)	0.6
Hemoglobin, g/L*	145 (112-171)	136 (94-166)	0.0001
Leukocyte count, x10^9/L	8.6 (3.9-17.1)	8.0 (3.1-16)	0.0002
Platelet count, x10^9/L*	702 (457-1451)	891 (480-2409)	0.0001
>1000x10^9/L, n (%)	22 (14)	93 (35)	<0.0001

**Median (range). M: male. F: female.*

Table 2. Reason for starting cytoreductive therapy in 433 patients with low-risk essential thrombocythemia.

	JAK2^{V617F} n= 74	*CALR* n=157
Age >60 years, n (%)	11 (15)	11 (7)
Thrombosis, n (%)	13 (18)	8 (5)
Bleeding, n (%)	5 (7)	5 (3)
Microvascular symptoms, n (%)	19 (26)	23 (15)
Extreme thrombocytosis, n (%)	18 (24)	98 (62)
Other, n (%)	8 (11)	12 (8)

JAK2^{V617F}*-mutated ET n=162,* CALR*-mutated ET n=271*

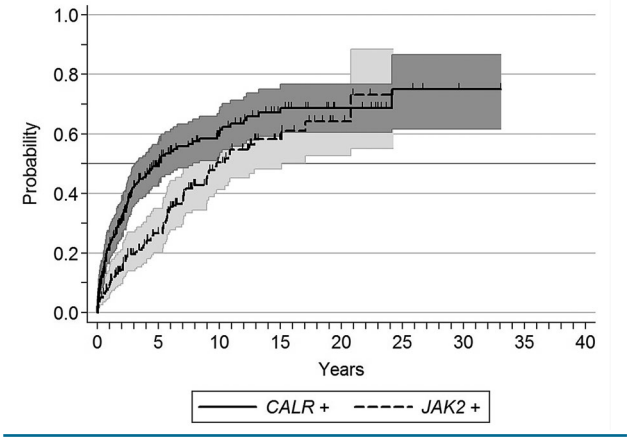

Figure 1. Time to cytoreductive therapy (95% confidence interval) according to genotype in low-risk ET. *P* value = 0.0002.

and cardiovascular risk factors had a significantly higher risk of thrombosis (incidence rate: 22 *versus* 8.9 events per 1000 patient-years in the remaining patients; *P*=0.04), which persisted after adjustment for treatment with low-dose aspirin (IRR: 2.5, 95% CI: 1.1-5.7, *P*=0.03). Coexistence of the *JAK2*^{V617F} mutation and cardiovascular risk factors increased the risk of arterial thrombosis (IRR: 3.2, 95% CI: 1.1-9.4, *P*=0.03) but had no effect on the incidence of venous thrombosis (IRR: 1.7, 95% CI: 0.5-6.7, *P*=0.4).

Patients with concomitant cardiovascular risk factors and leukocytosis showed a higher rate of arterial thrombosis than the remainder (incidence rate: 26.3 *versus* 4.5 events per 1000 patient-years, *P*=0.005). Adjusting by low-dose aspirin treatment showed a higher risk of arterial thrombosis in patients with both cardiovascular risk factors and leukocytosis (IRR: 6.2, 95% CI: 1.9-19.5, *P*=0.02) whereas treatment with aspirin did not result in a reduction of thrombotic risk (IRR 1.4, 95% CI: 0.4-4.6, *P*=0.5). When genotype was included in the multivariate model the increased risk associated with concomitant cardiovascular risk factors and leukocytosis was only observed in *JAK2*^{V617F}-mutated patients (IRR: 5.8, 95% CI: 1.3-26.9, *P*=0.023) but not in those with the *CALR* mutation (IRR: 4.9, 95% CI: 0.6-37.3, *P*=0.1)

Overall, 17 major bleeding episodes were registered, 13 while patients were on antiplatelet therapy and four while on careful observation (incidence rate: 9.9 and 4.6 events per 1000 person-years, respectively; *P*=0.2, Figure 5). No significant differences in the rate of major bleeding were observed in *JAK2*^{V617F}-positive patients according to type of therapy (Figure 5), whereas *CALR*-mutated patients experienced a higher rate of major bleeding while on antiplatelet therapy than on careful observation (12.9 *versus* 1.8 bleeding events per 1000 person-years, respectively, *P*=0.03, Figure 5).

Interaction analyses were performed to assess whether the risk of hemorrhage associated with the coexistence of antiplatelet therapy and marked thrombocytosis (>1000x10^9/L) varied with the *CALR/JAK2*^{V617F} genotype. In *CALR*-mutated patients, antiplatelet therapy was associated with a tendency to an increased risk of bleeding (IRR 6.9, 95% CI: 0.9-54.1, *P*=0.06) whereas extreme thrombocytosis was not (IRR: 2.7, 95% CI: 0.7-9.5, *P*=0.1). In contrast, in *JAK2*^{V617F}-mutated patients, extreme thrombocytosis was

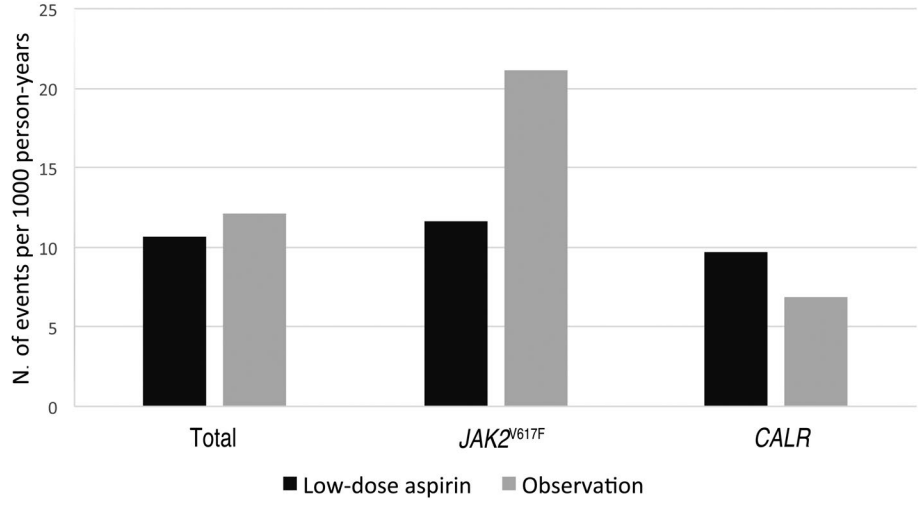

Figure 2. Incidence rate of thrombosis (arterial or venous) in ET patients treated with low-dose aspirin or careful observation. Rates according to therapy are provided for the whole cohort of patients (*P*=0.7), *JAK2*^{V617F}-mutated patients (*P*=0.2) and *CALR*-mutated patients (*P*=0.6).

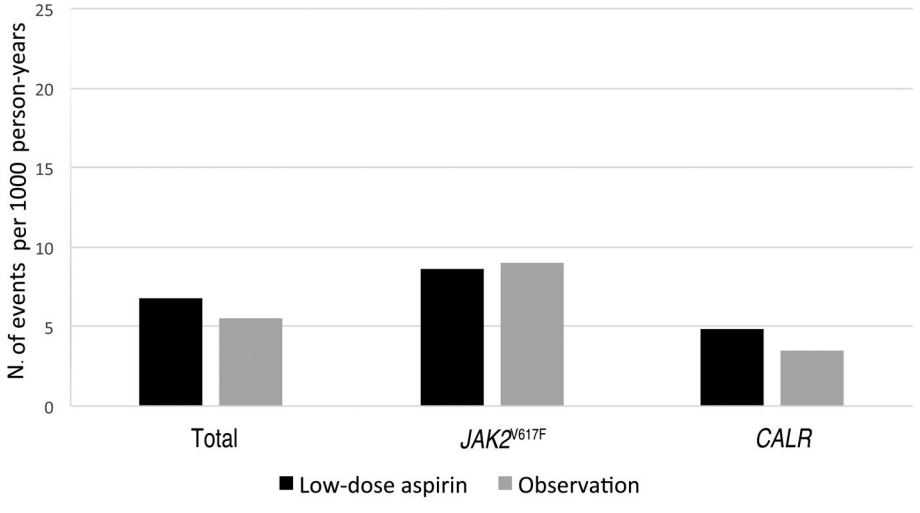

Figure 3. Incidence rate of arterial thrombosis in ET patients treated with low-dose aspirin or careful observation. Rates according to therapy are provided for the whole cohort of patients (*P*=0.7), *JAK2*^{V617F}-mutated patients (*P*=0.9) and *CALR*-mutated patients (*P*=0.7).

associated with an increased risk of bleeding (IRR: 9.8, 95% CI: 2.3-42.3, *P*=0.002) whereas antiplatelet therapy was not (IRR: 0.9, 95% CI: 0.2-3.3, *P*=0.9). Additional interaction analyses failed to demonstrate a significant association between leukocytosis and major bleeding even when antiplatelet therapy or genotype were included in the multivariate model.

Discussion

In this study, we retrospectively assessed the benefit/risk balance of antiplatelet therapy in low-risk ET during the period of time in which patients were free from cytoreduction. To the best of our knowledge, it represents the largest series of low-risk patients and the first one evaluating antiplatelet therapy in *CALR*-mutated ET. The main finding was the lack of reduction of thrombosis combined with a higher incidence of major bleeding in *CALR*-mutated patients when receiving low-dose aspirin. These results question the indication for monotherapy with antiplatelet agents in *CALR*-positive low-risk ET.

Recently, a new score including cardiovascular risk factors and *JAK2*^{V617F} mutational status, in addition to age and history of thrombosis, has been proposed to assess the risk of thrombosis in ET.[15] Application of this new score to patients with low-risk ET implies that both the *CALR/JAK2* genotype and cardiovascular risk factors should be taken into account in guiding therapeutic decisions.[16] In this regard, a previous study of 300 patients with low-risk ET showed that antiplatelet therapy was effective in reducing the incidence of venous thrombosis in patients with the *JAK2*^{V617F} mutation and arterial thrombosis in patients with cardiovascular risk factors.[5] The present study expands on these previous findings by revealing a synergistic effect between cardiovascular risk factors with both *JAK2*^{V617F} mutation and leukocytosis, resulting in an increased risk of arterial thrombosis and, more importantly, that antiplatelet therapy is not able to offset such increased risk.

Interaction analyses showed a higher risk of bleeding in *CALR*-mutated patients treated with low-dose aspirin which was independent of the platelet count. In contrast, in *JAK2*^{V617F}-mutated ET, the risk of bleeding was mostly associated with marked thrombocytosis, and was not influenced by antiplatelet therapy. This observation could be related to differences in platelet function according to the type of mutation. In this regard, recent studies showed

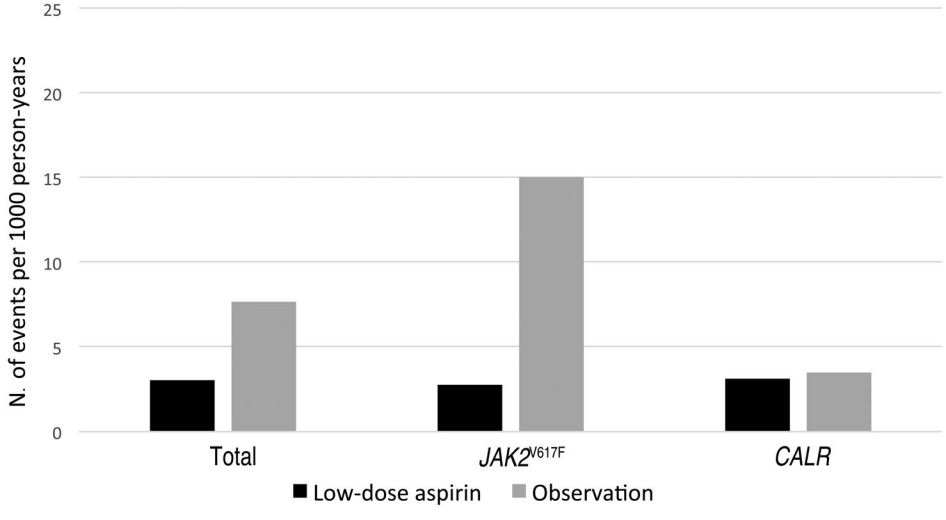

Figure 4. Incidence rate of venous thrombosis in ET patients treated with low-dose aspirin or careful observation. Rates according to therapy are provided for the whole cohort of patients (*P*=0.1), *JAK2*^{V617F}-mutated patients (*P*=0.045) and *CALR*-mutated patients (*P*=0.9).

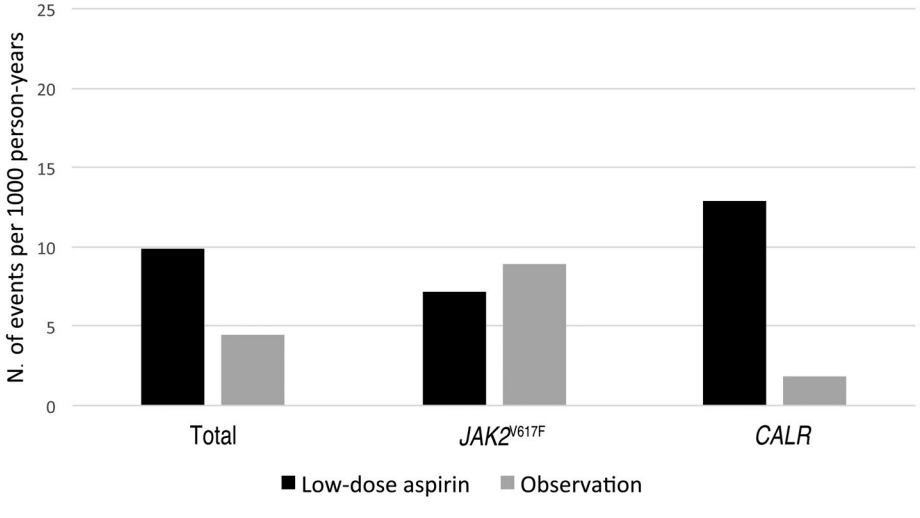

Figure 5. incidence rate of major bleeding in ET patients treated with low-dose aspirin or careful observation. Rates according to therapy are provided for the whole cohort of patients (*P*=0.17), *JAK2*^{V617F}-mutated patients (*P*=0.8) and *CALR*-mutated patients (*P*=0.03).

more pronounced platelet dysfunction and lesser platelet activation in patients carrying *CALR* mutations than in those with *JAK2*^{V617F}-positive ET, a feature that might result in a higher aspirin-induced bleeding diathesis.[17,18] Interestingly, patients with *CALR*-mutated ET were treated with cytoreductive therapy sooner after diagnosis (median 5 years compared to 9.8 years in *JAK2*^{V617F}-mutated cases), usually to control extreme thrombocytosis. This finding suggests that, in routine clinical practice, treatment needs in low-risk ET patients differ according to whether they have the *JAK2*^{V617F} or *CALR* mutation. Thus, while *JAK2*^{V617F}-mutated patients are usually managed in the long-term with antiplatelet therapy, this is not the case with their *CALR*-mutated counterparts, who appear to have a higher requirement for cytoreduction.

Current recommendations from experts adopt the same treatment algorithm for all low-risk ET patients, regardless of the *JAK2*^{V617F} and *CALR* genotype.[1,2] However, the results from the present study call for a distinctive, genotype-based therapeutic approach. Indeed, in *CALR*-mutated patients both the failure of antiplatelet agents to prevent thrombosis and the increased need for cytoreductive therapy using current algorithms suggest that such patients could benefit from an individualized therapeutic approach, different from that used in *JAK2*^{V617F}-mutated patients. Thus, in low-risk *CALR*-mutated ET, in which the incidence of thrombosis is very low, careful observation would be a reasonable option for asymptomatic patients, while in patients with symptoms or marked thrombocytosis, cytoreductive therapy would be preferable because of its efficacy and low associated risk of bleeding. On the contrary, in patients with *JAK2*^{V617F}-positive ET, antiplatelet therapy would be superior to abstention, providing an adequate antithrombotic effect without a definite increase in the risk of bleeding. Nevertheless, in patients with *JAK2*^{V617F} mutation with concomitant cardiovascular risk factors and/or leukocytosis, the antithrombotic effect of antiplatelet therapy may be insufficient. This subset of patients might be candidates for cytoreduction, especially in the presence of marked thrombocytosis.

The retrospective design of the present study is a limitation when drawing conclusions to guide clinical practice, because of the possibility of biases in patient selection and therapeutic decisions. Such drawbacks can be avoided with randomized clinical trials. Of particular interest would be trials comparing therapeutic abstention to antiplatelet therapy in *CALR*-mutated low-risk ET as well as those comparing the effects of aspirin *versus* cytoreduction in low-risk patients with *JAK2*^{V617F} mutation and cardiovascular risk factors. However, these studies require the inclusion of a large number of patients and a very long follow-up, which is highly costly and logistically complicated. Meanwhile, observational studies may help in defining a reasonable treatment strategy.

In conclusion, in patients with low-risk *CALR*-mutated ET, antiplatelet therapy with low-dose aspirin does not reduce the frequency of thrombosis and may increase the incidence of bleeding.

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