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RESEARCH ARTICLE

# Long-term pharmacodynamic and clinical effects of twice- versus once-daily low-dose aspirin in essential thrombocythemia: The ARES trial

Bianca Rocca[1,2] | Alberto Tosetto[3] | Giovanna Petrucci[1,4] | Elena Rossi[4,5] | Silvia Betti[5] | Denise Soldati[5] | Alessandra Iurlo[6] | Daniele Cattaneo[6,7] | Cristina Bucelli[6] | Alfredo Dragani[8] | Mauro Di Ianni[8,9] | Paola Ranalli[8,9] | Francesca Palandri[10] | Nicola Vianelli[10] | Eloise Beggiato[11] | Giuseppe Lanzarone[11] | Marco Ruggeri[3] | Giuseppe Carli[3] | Elena Maria Elli[12] | Rossella Renso[12] | Maria Luigia Randi[13] | Irene Bertozzi[13] | Giuseppe Gaetano Loscocco[14] | Alessandra Ricco[15] | Giorgina Specchia[16] | Alessandro M. Vannucchi[14] | Francesco Rodeghiero[17] | Valerio De Stefano[4,5] | Carlo Patrono[1,18] | for the Aspirin Regimens in EsSential thrombocythemia (ARES) Investigators

[1]Department of Safety and Bioethics, Section of Pharmacology, Catholic University School of Medicine, Rome, Italy

[2]Department NeuroFarBa, University of Florence, Florence, Italy

[3]Hematology Department, San Bortolo Hospital, Vicenza, Italy

[4]Department of Radiological and Hematological Sciences, Section of Hematology, Catholic University School of Medicine, Roma, Italy

[5]Fondazione Policlinico Universitario A. Gemelli, IRCCS, Roma, Italy

[6]Hematology Division, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy

[7]Department of Oncology and Hemato-Oncology, University of Milan, Milano, Italy

[8]Hematology Unit, Department of Oncology and Hematology, Ospedale Civile "Santo Spirito", Pescara, Italy

[9]Department of Medicine and Aging Sciences, "G. D'Annunzio" University, Chieti, Italy

[10]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Policlinico S. Orsola-Malpighi, Istituto di Ematologia "Seràgnoli", Bologna, Italy

[11]Department of Oncology, Division of Hematology, A.O.U. Città della Salute e della Scienza di Torino, Torino, Italy

[12]Division of Haematology and Bone Marrow Transplantation Unit, Fondazione IRCCS San Gerardo Dei Tintori, Monza, Italy

[13]Department of Medicine—DIMED, University of Padova, Padova, Italy

[14]Department of Experimental and Clinical Medicine, CRIMM, Center of Research and Innovation of Myeloproliferative Neoplasms, Azienda Ospedaliero-Universitaria Careggi, University of Florence, Florence, Italy

[15]Unit of Hematology and Stem Cell Transplantation, AOU Consorziale Policlinico, Bari, Italy

[16]University of Bari Aldo Moro, Bari, Italy

[17]Hematology Project Foundation, Affiliated to the Department of Hematology, San Bortolo Hospital, Vicenza, Italy

[18]Center of Excellence on Aging, "G. D'Annunzio" University School of Medicine, Chieti, Italy

Prof Rocca, Dr Tosetto, Prof De Stefano, and Prof Patrono contributed equally to this article.

**Correspondence**
Valerio De Stefano, Section of Hematology, Department of Radiological and Hematological Sciences, Catholic University School of Medicine, Fondazione Policlinico A. Gemelli IRCCS, Largo Gemelli 9, 00168 Roma, Italy.
Email: valerio.destefano@unicatt.it

Bianca Rocca, Section of Pharmacology, Catholic University School of Medicine, Largo F. Vito 1, 00168, Rome, Italy.
Email: bianca.rocca@unicatt.it, b.rocca@tiscali.it

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## Abstract

Patients with essential thrombocythemia (ET) are treated with once-daily low-dose aspirin to prevent thrombosis, but their accelerated platelet turnover shortens the antiplatelet effect. The short-term Aspirin Regimens in EsSential Thrombocythemia trial showed that twice-daily aspirin dosing restores persistent platelet thromboxane (TX) inhibition. However, the long-term pharmacodynamic efficacy, safety and tolerability of twice-daily aspirin remain untested. We performed a multicenter, randomized, open-label, blinded-endpoint, phase-2 trial in which 242 patients with ET were randomized to 100 mg aspirin twice- or once-daily and followed for 20 months. The primary endpoint was the persistence of low serum $TXB_2$, a surrogate biomarker of antithrombotic efficacy. Secondary endpoints were major and clinically relevant non-major bleedings, serious vascular events, symptom burden assessed by validated questionnaires, and in vivo platelet activation. Serum $TXB_2$ was consistently lower in the twice-daily versus once-daily regimen on 10 study visits over 20 months: median 3.9 ng/mL versus 19.2 ng/mL, respectively; $p < .001$; 80% median reduction; 95% CI, 74%–85%. No major bleeding occurred. Clinically relevant non-major bleedings were non-significantly higher (6.6% vs. 1.7%), and major thromboses lower (0.8% vs. 2.5%) in the twice-daily versus once-daily group. Patients on the twice-daily regimen had significantly lower frequencies of disease-specific symptoms and severe hand and foot microvascular pain. Upper gastrointestinal pain was comparable in the two arms. In vivo platelet activation was significantly reduced by the twice-daily regimen. In patients with ET, twice-daily was persistently superior to once-daily low-dose aspirin in suppressing thromboxane biosynthesis and reducing symptom burden, with no detectable excess of bleeding and gastrointestinal discomfort.

## 1 | INTRODUCTION

Essential thrombocythemia (ET) is a myeloproliferative neoplasm characterized by increased platelet generation and activation, and enhanced risk of thrombosis and hematological transformation.[1,2] Once-daily, low-dose aspirin (75–100 mg) is currently recommended for most ET patients to reduce the risk of thrombosis,[3] based on retrospective observational studies[4,5] and extrapolation from a randomized trial in polycythemia vera.[6] However, while most patients with polycythemia vera display adequate antiplatelet response to once-daily low-dose aspirin,[7,8] the majority of ET patients do not due to faster recovery of platelet cyclooxygenase (COX)-1 activity secondary to accelerated platelet generation.[9] This, in turn, results in higher platelet thromboxane (TX)$A_2$ production, as reflected by higher serum $TXB_2$ levels, particularly during the 12- to 24-h dosing interval.[10]

The Aspirin Regimens in ESsential Thrombocythemia (ARES) Investigators previously examined the relationship between the length of the aspirin dosing interval and the adequacy of the antiplatelet response by randomizing 252 aspirin-treated ET patients to a two-week treatment with aspirin 100 mg given once-, twice- or three times daily in a double-blind, placebo-controlled, short-term trial.[11,12] The results of this study identified twice-daily aspirin as the optimal dosing regimen to maximize inhibition of platelet $TXA_2$ production without interfering with vascular prostacyclin biosynthesis,[12] an important mediator of endothelial thromboresistance.[13] However, the persistence of more profound platelet TX suppression by more frequent aspirin dosing, long-term compliance with, and safety of this antiplatelet strategy are unknown.

The present phase-2 trial aimed to assess the long-term pharmacodynamic superiority (primary endpoint) and describe disease-related symptoms, tolerability, adherence, and safety of a twice-daily 100-mg aspirin regimen compared to the standard once-daily regimen in aspirin-treated ET patients.

## 2 | METHODS

### 2.1 | Study design

We conducted a randomized, open-label, blinded-endpoint, 20-month trial to compare the pharmacodynamic and clinical effects of twice-daily and once-daily 100-mg aspirin regimens. Patients were enrolled at 10 Italian hematological centers (Supplemental Material). The trial was registered on the EU clinical trial register as EudraCT 2016–

002885-30. The Ethics Committee of the Fondazione Policlinico Universitario A. Gemelli Istituto di Ricerca e Cura a Carattere Scientifico (IRCCS) approved the study (Protocol #28371/16, ID 1285). All participating institutions approved the protocol. All patients provided written informed consent before enrollment. The protocol is included in the Supplemental Material.

## 2.2 | Patients

Eligible patients had to meet the diagnostic criteria for ET,[14] be ≥18-year old and on low-dose aspirin prophylaxis since at least 1 month. Patients were diagnosed according to the 2016 WHO criteria[14]; diagnoses made before 2016 were reviewed for consistency with the 2016 WHO criteria at study entry, in order to exclude patients with pre-myelofibrosis due to their increased bleeding tendency.[15] The main exclusion criteria were: platelet number >1000 $\times$ $10^9$/L based on studies reporting a high bleeding risk associated with extreme thrombocytosis,[2] history of major bleeding, and need for chronic use of non-steroidal anti-inflammatory drugs (NSAIDs). The study protocol details all inclusion and exclusion criteria (Supplemental Material). These criteria allowed the inclusion of most patients who had previously participated in the two-week aspirin dose-finding study at the same centers.[12]

The following data were recorded in the electronic clinical report form at baseline: cardiovascular risk factors (diabetes, hypertension, obesity [defined as a body-mass index (BMI) ≥30 kg/$m^2$], current cigarette smoking, and dyslipidemia), *JAK2*, *CALR*, and *MPL* gene mutational status as analyzed at each center, and history of arterial or venous thrombosis. At each study visit, complete blood count, liver and kidney chemistry, new comorbidity and medication, thrombotic and hemorrhagic events (major, clinically relevant non-major, or minor), cancer, hematological transformation, and major or minor adverse events were recorded (see Study Protocol in the Supplemental Material). Clinical and laboratory characteristics were collected through Research Electronic Data Capture (REDCap).[16] The information and data recorded at baseline and subsequent study visits are further detailed in the Study Protocol.

Compliance was assessed by patient's self-reporting of drug intake, as recorded in her/his diary at each study visit and based on the preceding 3 months of therapy. Patients reported the daily timing of each tablet intake, any drug other than their usual therapy, and any symptom or comment they deemed relevant (see Supplemental Material). Although NSAIDs were prohibited during the study, occasional intake was carefully verified in the 3 days before each visit to exclude a drug–drug interaction potentially affecting serum $TXB_2$ measurements.[17]

## 2.3 | Randomization and masking

Patients were randomized to twice-daily low-dose aspirin (Cardioaspirin® 100 mg, Bayer Italy), to be taken at breakfast (7–9 am) and dinner (7–9 pm), or once-daily at breakfast only. Randomization was stratified by center and sex using a four-block randomization list.

The authors who performed prostanoid measurements and data analyses were blinded to the randomized treatment.

## 2.4 | Procedures

Patients underwent study visits three times within the first 5 ± 1 weeks and then every 3 months over a 20-month follow-up. Blood and urine samples were collected in the morning, in a fasting state, immediately before aspirin dosing.

### 2.4.1 | Pharmacodynamic and laboratory measurements

Pharmacodynamic efficacy was assessed by centralized blinded measurement of serum $TXB_2$,[18] the primary endpoint of the study, that was repeated 10 times over a 20-month follow-up after randomization (see Supplemental Material). The assessment of pre-analytical variables and the reproducibility of serum $TXB_2$ measurements in the ARES centers have been previously described.[11] In vivo platelet activation was assessed by the urinary excretion of 11-dehydro-$TXB_2$, a major enzymatic metabolite of $TXA_2$/$TXB_2$.[19,20]

### 2.4.2 | Patient-reported symptoms

The severity of all disease-specific symptoms was graded by the patient at each visit using the validated Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF), which considers a 0–10 semiquantitative visual scale.[21] We subsequently summarized the severity of the symptoms burden into a single validated measure, the MPN-SAF total symptoms score (referred to as MPN-SAF-TSS or MPN-10) that sums the scores of 10 symptoms of the MPN-SAF questionnaire (fatigue, early satiety, abdominal discomfort, inactivity, concentration problems, night sweats, itching, bone pain, fever, and unintentional weight loss) and ranges from 0 to 100.[22,23] Microvascular symptoms of hands and feet (erythromelalgia), were assessed by self-completed questionnaires with a numerical pain rating scale (NRS; see Supplemental Material).[24] Gastrointestinal symptoms were evaluated according to the Severity of Dyspepsia Assessment (SODA) questionnaires,[25] based on numerical rating scales specifically related to stomach pain and upper gastrointestinal discomfort experienced during the week preceding the study visit. Questionnaires were available for SODA1 (a number from 0 to 100 describing the symptom of pain in the upper abdomen) and SODA2 (a number from 0 to 10 describing the symptom of discomfort in the upper abdomen).

## 2.5 | Endpoints

The primary endpoint of the study was the long-term persistence of superior pharmacodynamic efficacy, as reflected by serum $TXB_2$, of the twice-daily as compared to the once-daily aspirin regimen.

Overall, 2556 peripheral venous blood samples were collected and analyzed, with 4% of planned blood sampling missed because of COVID-19-related restrictions or other causes. A total of 81 serum samples with serum $TXB_2$ values below the lowest analytical threshold (0.5 ng/mL) and 5 samples with documented pre-analytical handling errors were excluded, leaving 2470 serum samples available for the statistical analyses (1244 and 1226 in the once- and twice-daily treatment arms, respectively; Supplemental Table 1).

Secondary endpoints were: (1) major arterial and venous thrombotic complications, that is, ischemic stroke, transient ischemic attack (TIA), acute coronary syndrome, peripheral artery thrombosis, deep vein thrombosis, pulmonary embolism, cerebral and splanchnic vein thrombosis, or revascularization (see Study Protocol); (2) symptom burden and treatment tolerability, as described above (MPN-10, NRS for microvascular symptoms of hands and feet [erythromelalgia] as well as SODA-1 and SODA-2 questionnaire)[21–25]; (3) in vivo platelet activation, as reflected by measurements of urinary 11-dehydro-$TXB_2$ excretion.

Safety was evaluated by reporting major and clinically relevant non-major bleedings according to the International Society on Thrombosis and Haemostasis classification[26,27] and any other adverse events considered attributable to aspirin by the responsible physician (Study Protocol in the Supplemental Material).

## 2.6 | Statistical analyses

To test the hypothesis of a persistent superiority of the twice- versus the once-daily aspirin dosing regimen, 112 patients per arm were needed to demonstrate, with $\alpha$ and $\beta$ errors of .05 and .80, respectively, a relative reduction by at least 50% in serum $TXB_2$ with the experimental versus the standard aspirin regimen, in at least 60% of the 10 planned study visits (Study Protocol in the Supplemental Material).

Data were analyzed on an intention-to-treat basis; no replacement for missing values was planned, and no interim or exploratory analyses were carried out. The primary endpoint was evaluated by comparing the median serum $TXB_2$ in the two treatment groups across all post-randomization visits, using a non-parametric Wilcoxon test with Scheffe's adjustment for multiple comparisons. Differences between frequencies of secondary endpoints (e.g., the frequency of MPN-10 or NRS scores in the two groups) were tested by chi-square statistics, as detailed in the Study Protocol (Supplemental Material).

For subgroup analyses, the serum $TXB_2$ change was first computed for each participant as the ratio between the median $TXB_2$ value across all post-randomization visits and the baseline serum $TXB_2$. We then calculated the ratios between the median change in patients randomized to the aspirin twice- versus once-daily regimen in each subgroup. Quantile regression with Scheffe's multiple-comparison correction was used to test for interaction. Ninety five percent confidence intervals were calculated by bootstrapping. The analysis of the SODA 1 and SODA 2 questionnaires is described in the Supplemental Material.

Post-hoc analyses included: (1) evaluation of the consistency of intra-subject coefficients of variation of serum $TXB_2$, as calculated in the first 3 months (visit 2 to 5), between 4 and 12 months (visit 6 to 8), and between 13 and 18 months (visit 9 to 11); (2) subgroup analyses based on age, sex, BMI, platelet count, JAK2 mutational status, cytoreduction, previous thrombosis, number of arterial risk factors, and disease duration; (3) correlation between individual measurements of serum $TXB_2$ and urinary 11-dehydro-$TXB_2$.

The R 4.2.1 (The R Foundation for Statistical Computing, Vienna, Austria) was used for all data analyses and plotting; the package *ComplexUpset* was used for upset plots.[28]

## 2.7 | Role of the funding source

The Italian Medicines Agency (AIFA) funded the study (Study FARM12Y8HH), and the trial medication was provided by Bayer AG free of charge. The funding agency and Bayer AG had no role in the study design, data analysis, and interpretation. The manuscript was drafted by BR, AT, VDS, and CP, and reviewed by all the authors. All the authors decided to submit the manuscript for publication and vouch for the completeness and accuracy of the data and analyses and for the fidelity of the study to the protocol.

# 3 | RESULTS

## 3.1 | Study participants

The first patient was enrolled on November 14, 2018, the last patient on March 30, 2019. The study ended on February 23, 2021. Two hundred and forty two patients were randomized to aspirin 100 mg once-daily (n = 120) or twice-daily (n = 122). The number of patients who completed the study and the reasons for study discontinuation are shown in Supplemental Figure 1. Two hundred and twenty two out of 242 randomized patients (92%) completed all planned clinical and laboratory evaluations; 10 patients completed half or less of the scheduled visits. Twenty four visits were performed online because of COVID-19 pandemics.

The demographic and clinical characteristics of the patients at baseline were similar in the two treatment groups (Table 1). The median age was 61 years, 46% were males, the median duration of ET was 6 years, the median platelet count was $528 \times 10^9$/L, and a previous thrombosis had occurred in 26 patients. All patients had been on once-daily low-dose aspirin for at least 1 month for either primary (89%) or secondary (11%) cardiovascular prophylaxis, according to current recommendations,[1,2,29] and over half were on cytoreductive treatment with hydroxyurea.

Median treatment adherence to therapy, based on self-reporting, was 99% in both groups for the whole study duration.

## 3.2 | Pharmacodynamic primary endpoint

During a 20-month follow-up, the level of serum $TXB_2$ was significantly lower in patients on the twice- versus once-daily aspirin

**TABLE 1** Baseline characteristics of patients with essential thrombocythemia.

| | All patients *N* = *242* | Aspirin 100 mg once-daily *N* = *120* | Aspirin 100 mg twice-daily *N* = *122* | *p* overall |
|---|---|---|---|---|
| Sex: | | | | .99 |
| Female—no. (%) | 130 (54) | 65 (54) | 65 (53) | |
| Male—no. (%) | 112 (46) | 55 (46) | 57 (47) | |
| Age, year | 61 [51;68] | 60 [50;69] | 61 [52;68] | .66 |
| Duration of disease, year | 6 [3;10] | 5 [3;10] | 6 [4;10] | .25 |
| Age at diagnosis, year | 54 [42;63] | 53 [43;64] | 54 [42;63] | .98 |
| Body mass index, kg/m$^2$ | 25 [23;27] | 25 [23;28] | 25 [23;27] | .68 |
| Hematocrit, % | 42 [39;46] | 42 [39;46] | 42 [39;46] | .88 |
| Leukocytes, 10$^9$/L | 7 [5.6;8] | 7 [6;8] | 7 [5;8] | .18 |
| Platelets, 10$^9$/L | 528 [440;670] | 558 [440;694] | 525 [437;654] | .54 |
| JAK V617F—no. (%) | | | | .21 |
| WT | 93 (38) | 41 (34) | 52 (43) | |
| Mutated | 148 (61) | 78 (65) | 70 (57) | |
| Not done | 1 (0.4) | 1 (0.8) | 0 | |
| CALR—no. (%) | | | | .04 |
| WT | 81 (34) | 41 (35) | 40 (33) | |
| Type1 | 20 (8) | 14 (12) | 6 (5) | |
| Type2 | 16 (7) | 3 (3) | 13 (11) | |
| Other | 6 (3) | 3 (3) | 3 (2) | |
| Not done | 119 (49) | 59 (49) | 60 (49) | |
| MPL—no. (%) | | | | .81 |
| WT | 109 (45) | 51 (43) | 58 (47) | |
| Mutated | 4 (2) | 2 (2) | 2 (2) | |
| Not done | 129 (53) | 67 (55) | 62 (51) | |
| Cytoreduction—no. (%) | | | | .53 |
| No | 93 (38) | 49 (41) | 44 (36) | |
| Yes | 149 (62) | 71 (59) | 78 (64) | |
| Hydroxyurea—no. (%) | | | | .90 |
| No | 119 (49) | 60 (50) | 59 (48) | |
| Yes | 123 (51) | 60 (50) | 63 (52) | |
| Anagrelide—no. (%) | | | | .85 |
| No | 222 (92) | 111 (92) | 111 (91) | |
| Yes | 20 (8) | 9 (7) | 11 (9) | |
| Previous thrombosis: MPN-related[a]—no. (%) | 10 (4) | 5 (4) | 5 (4) | 1.00 |
| Any thrombosis—no. (%) | 26 (11) | 13 (11) | 13 (11) | 1.00 |
| Risk factors—no. (%) | | | | |
| Diabetes | 14 (6) | 7 (6) | 7 (6) | 1.00 |
| Hypertension | 84 (35) | 40 (33) | 44 (36) | .76 |
| Obesity (BMI ≥ 30 kg/m$^2$) | 31 (13) | 19 (16) | 12 (10) | .22 |
| Cigarette smoking | 37 (15) | 14 (12) | 23 (19) | .17 |
| Dyslipidemia | 54 (22) | 30 (25) | 24 (20) | .40 |
| IPSET score[53]—no. (%) | | | | .73 |
| 0 | 27 (11) | 12 (10) | 15 (12) | |
| 1 | 38 (16) | 18 (15) | 20 (16) | |
| 2 | 70 (29) | 37 (31) | 33 (27) | |
| 3 | 50 (21) | 26 (22) | 24 (20) | |

**TABLE 1** (Continued)

| | All patients $N = 242$ | Aspirin 100 mg once-daily $N = 120$ | Aspirin 100 mg twice-daily $N = 122$ | $p$ overall |
|---|---|---|---|---|
| 4 | 43 (18) | 18 (15) | 25 (20) | |
| 5 | 5 (2) | 4 (3) | 1 (1) | |
| 6 | 9 (4) | 5 (4) | 4 (3) | |

[a]Thrombosis occurred up to 2 years before diagnosis of MPN. Data are *n* (%) or median (range) as indicated.

regimen (median, 3.9 [IQR, 2.3–6.0] vs. 19.2 [IQR, 10.2–37.7] ng/ml; $p < .001$), corresponding to a median 80% reduction (95% confidence interval, 74%–85%), with no evidence of time-related attenuation of such difference between the two dosing regimens (Figure 1). The intra-subject coefficient of variation of three repeated measurements of serum $TXB_2$ was similar in the two groups and did not change with treatment duration (Supplemental Table 2).

As shown in Supplemental Figure 2, a large difference in serum $TXB_2$ between the twice-daily and once-daily aspirin regimens was consistently measured in all sub-groups, including patients with higher platelet counts, with risk factors (i.e., age > 60 years, previous thrombosis, presence of the JAK2V617F mutation, other known cardiovascular risk factors), and those not receiving cytoreductive therapy.

## 3.3 | Secondary endpoints

### 3.3.1 | Symptom burden, thrombotic outcomes, and in vivo platelet activation

Over the 20-month study period, a total of 2561 MPN-SAF questionnaires were available for statistical analyses (1285 and 1276 from patients on once- and twice-daily aspirin, respectively). As shown in Supplemental Table 3, the mean score values of each symptom contributing to the MPN-10 score were significantly lower in the twice- versus once-daily regimen groups. Figure 2 shows that patients in the twice-daily regimen group had a higher number of questionnaires at each study visit with no reported symptoms ($n = 221$, 17.3%) as compared to patients in the once-daily regimen group ($n = 184$, 14.3%, $p = .05$). On the contrary, eight or more symptoms were reported in 101 (7.9%) and 167 questionnaires (13.0%, $p = .0001$) by patients on the twice- and once-daily regimen, respectively. There was a statistically significant difference in the frequency of MPN-10 (Figure 3, upper panel) and NRS hands and feet pain scores (Figure 3, middle and lower panel), with less severe scores being recorded more frequently in patients assigned to receive twice-daily aspirin than in those assigned to the standard once-daily regimen. For peripheral microvascular pain of both hands and feet, the differences were most apparent in the higher categories of the numerical rating scale (≥2) at each study visit (Figure 3, middle and lower panel). Patients taking twice-daily aspirin reported a pain category ≥2 significantly less frequently than those taking once-daily aspirin, for both hands (19% vs. 25%, respectively; $p < .001$) and feet (20% vs. 27%, respectively; $p < .001$).

A low number of major thrombotic complications was recorded in the whole study, with 1 and 3 events in patients on the twice- and once-daily regimens, respectively (Supplemental Table 4). The number of microvascular events (headache, dizziness, acroparesthesia, acromelalgia) of sufficient intensity to be reported by the attending physicians as a clinically relevant adverse event was significantly lower in patients randomized to the twice-daily regimen as compared to those in the once-daily arm ($n = 17$ [13.9%], vs. $n = 31$ [25.8%], $p = .02$; respectively, Supplemental Table 4).

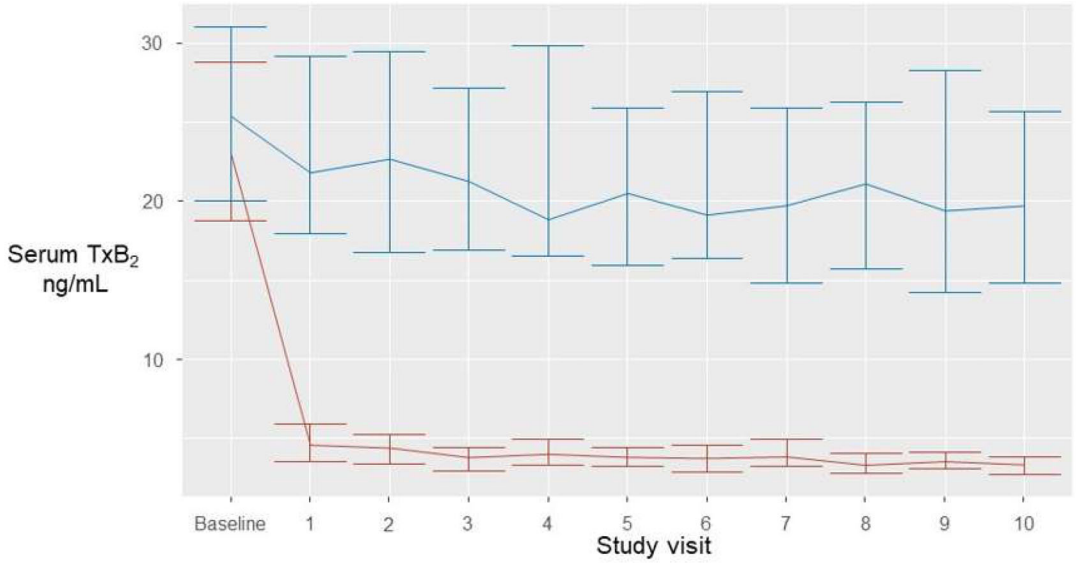
In vivo platelet activation, as assessed by urinary 11-dehydro-$TXB_2$ excretion,[30] was significantly and persistently lower in patients on the twice-daily versus those on the once-daily aspirin regimen (median, 171 versus 301 pg/mg creatinine, respectively; $p < .001$), corresponding to a median 43% relative reduction (Supplemental Figure 3). A statistically significant correlation was observed between individual paired measurements of serum $TXB_2$ and urinary 11-dehydro-$TXB_2$ (rho = .46, $p < .001$; Figure 1), suggesting that less-than-complete inhibition of platelet COX-1 activity is an important determinant of persistent $TXA_2$-dependent platelet activation in vivo.

### 3.3.2 | Safety

No major bleeding occurred during the 20-month follow-up. Clinically relevant non-major bleedings were numerically, but non-significantly, higher (8 vs. 2) in the experimental group than in the control group (Supplemental Table 4). Minor bleedings were reported by five and four patients, respectively. None of the patients with CARL mutation had clinically relevant non-major bleeding events. The low number of events did not allow subgroup analyses comparing different mutational profiles, but in patients with platelet counts $<1000 \times 10^9$/L the CALR mutation was not associated with higher bleeding, unlike previously observational and retrospective studies.[4]

The frequency of upper gastrointestinal pain and discomfort, self-assessed by using the SODA questionnaires, was available in 240 out of 242 patients. The median SODA scores throughout the study were comparable in the two study groups (median SODA pain score, 0 in both groups; median SODA discomfort score, 1.0 in both groups; Supplemental Figure 4).

Other adverse events that occurred during the study (Supplemental Table 5) were considered by the referent physicians unrelated to the experimental treatment.




| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 100 mg once daily, n | 120 | 120 | 118 | 116 | 118 | 115 | 115 | 112 | 93 | 108 | 109 |
| 100 mg twice daily, n | 122 | 116 | 117 | 116 | 116 | 116 | 114 | 110 | 89 | 103 | 108 |
| $TxB_2$ Difference, % | - 9.0 | -79.0 | -80.7 | -82.1 | -78.7 | -81.4 | -80.4 | -80.6 | -84.4 | -81.8 | -83.1 |
| P | 0.466 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

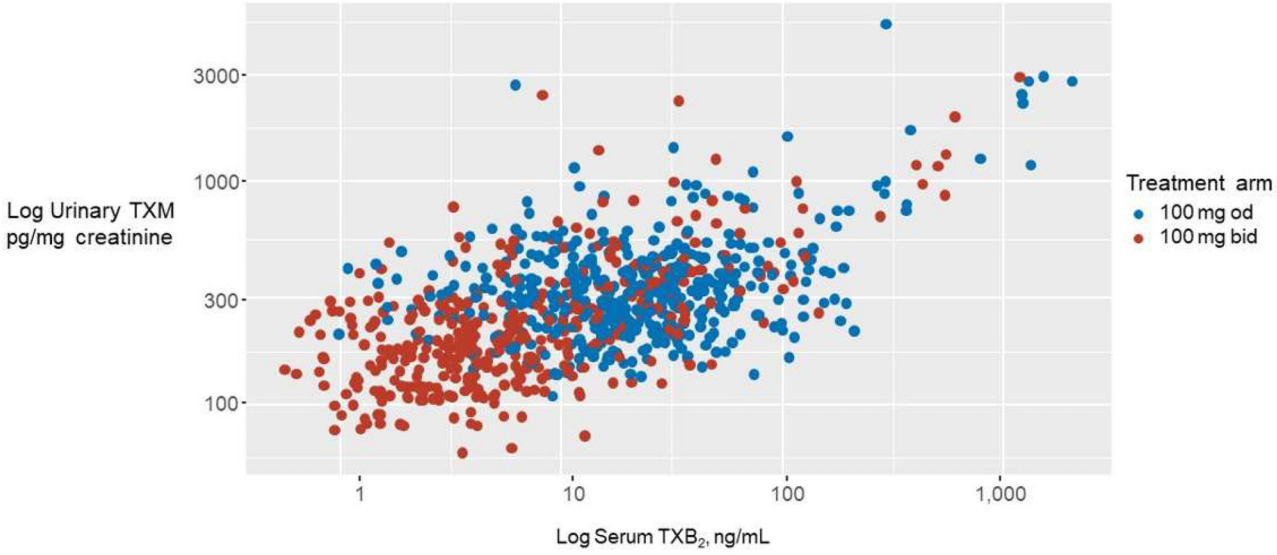



**FIGURE 1** Serum thromboxane $B_2$ values according to the randomized aspirin regimen. In the upper panel the figure depicts the median serum thromboxane (TX) $B_2$ at randomization (baseline) and during the 10 post-randomization study visits in the two treatment arms. For each time-point, error bars represent the upper and lower 95% confidence interval (CI) around the median; the table below reports the number of valid measurements, serum $TXB_2$ percent reduction for the twice-daily versus once-daily regimen, and *p* value after Scheffe's correction. The lower panel depicts the correlation between serum thromboxane B2 and urinary thromboxane metabolite excretion in ET patients treated with two aspirin regimens. The plot shows individual data from 846 visits in which both serum thromboxane (TX) B2 and urinary TXA2/TXB2 metabolite (TXM) were measured in the 242 enrolled ET patients, randomized to receive aspirin 100 mg od or bid.

## 4 | DISCUSSION

The natural history of ET is characterized by an increased incidence of thrombotic and microvascular complications.[1,2] In the largest available population-based cohort study of 9429 MPN patients and 35 820 matched control participants, the rate of thromboses (arterial and venous) in 3462 ET patients was between 1.7- and 3.5-fold higher than in the age- and gender-matched population, depending on the age of onset and time from diagnosis.[31]

Low-dose aspirin (81 to 100 mg daily) is considered—largely by consensus opinion—the cornerstone of antithrombotic therapy for patients with ET.[2] While randomized trials of aspirin in this setting are lacking, the potential benefit for ET was extrapolated from the positive results of a placebo-controlled, low-dose aspirin trial involving

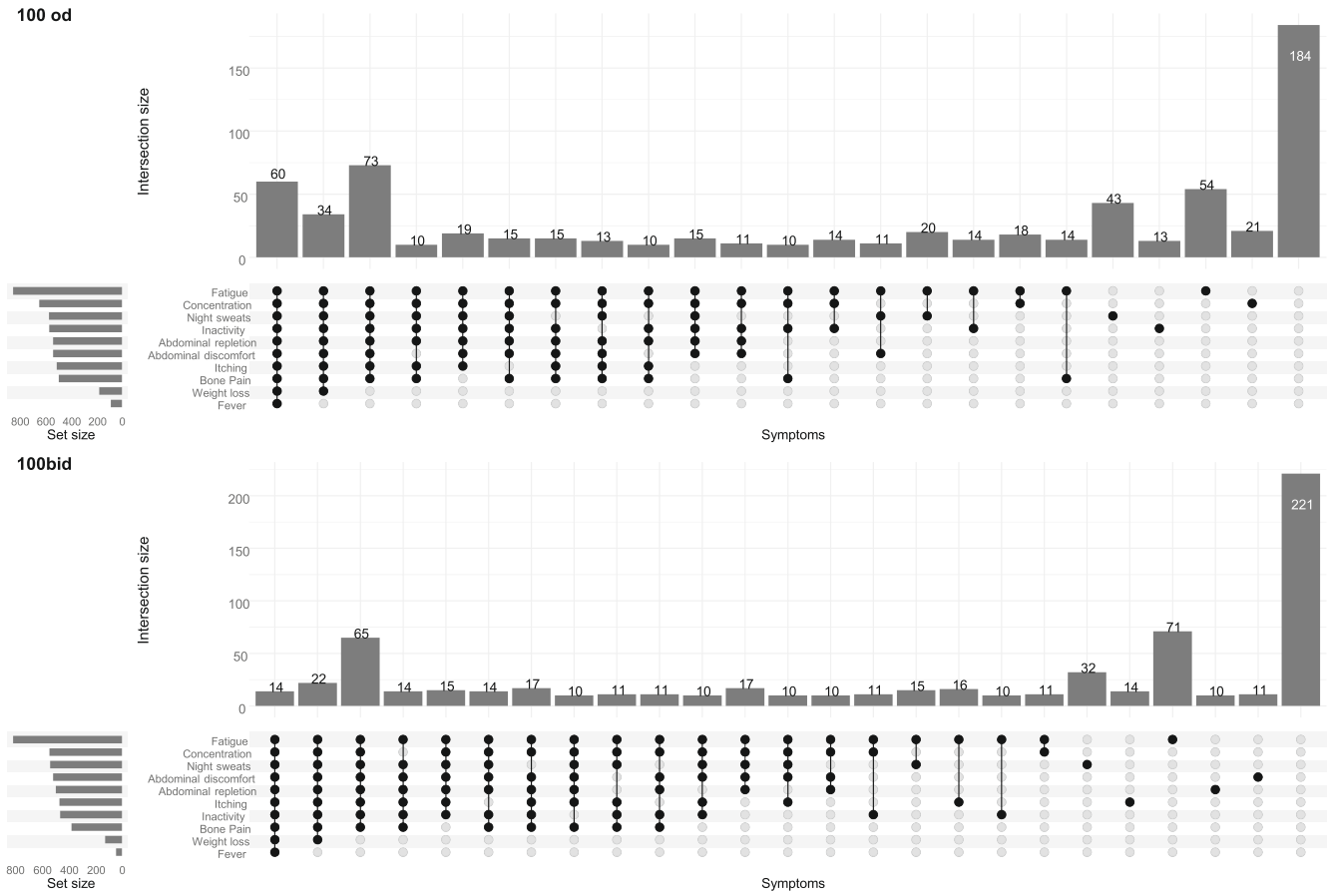



**FIGURE 2** Distribution of the MPN-10 disease-specific symptom score according to the randomized aspirin regimen. In the upset plot, the main vertical bars represent the absolute number of visits in which symptoms clustered together as represented by the black connected dots underneath the bars. The horizontal bars (left side) represent the relative frequency of each symptom. Upper and lower panels show data for the once- and the twice-daily regimens, respectively. As an example, patients randomized to once-daily aspirin referred a complete absence of any symptom in 184 visits, as compared to 221 visits in patients taking twice-daily aspirin. On the other hand, patients randomized to once-daily aspirin complained of suffering from all 10 possible symptoms in 60 visits, as compared to 14 visits in patients taking twice-daily aspirin.

518 patients with polycythemia vera[6] and retrospective studies.[4,5,32] Therefore, the efficacy and safety of antiplatelet therapy in ET remains untested.[32] However, it should be acknowledged that the rarity of the disease and the relatively low vascular event rate would make a cardiovascular outcome trial in essential thrombocytemia unfeasible.

Although the assessment of platelet aggregation played a pivotal role in the development of antiplatelet drugs,[33] measurement of serum $TXB_2$ was instrumental in the development of low-dose aspirin as an antiplatelet agent, as it guided the choice of dosing regimens for randomized clinical trials to evaluate its efficacy and safety.[34] According to the European Medicines Agency, "the reduction of $TXB_2$ levels in serum (not in plasma) can be considered as a widely accepted surrogate for platelet aggregation and also for efficacy in secondary prevention of cardiovascular events"[20] (Supplemental material). Similarly, the Food and Drug Administration (FDA) included serum $TXB_2$ measurement as part of the new drug approval (NDA) file for a novel liquid formulation of aspirin.[35]

Because of the very high capacity of human platelets to synthesize and release $TXA_2$ in response to various stimuli, platelet COX-1 activity must be virtually completely (i.e., >97%) suppressed to maximally reduce platelet activation in vivo, as reflected by urinary 11-dehydro-$TXB_2$ excretion.[36,37] Such a profound and long-lasting suppression of serum $TXB_2$ can be achieved with a once-daily regimen of 75- to 100-mg aspirin, but not with other NSAIDs because of their time-dependent, reversible inhibition of platelet COX-1 activity.[38] Incomplete (i.e., 70% to 90%) and short-lasting reductions in serum $TXB_2$ by ibuprofen or diclofenac were not associated with any measurable cardioprotective effect and did not prevent atherothrombotic complications associated with COX-2 inhibition.[39] In light of these findings, it is pharmacologically plausible that a conventional low-dose aspirin regimen producing a similarly incomplete reduction in platelet $TXA_2$ production will be largely ineffective in protecting patients with ET against atherothrombosis.[40]

The ARES Investigators previously reported that the majority of ET patients display inadequate suppression of serum $TXB_2$ by a standard low-dose aspirin regimen,[12] most likely due to accelerated renewal of platelet COX-1 during the 24-h dosing interval.[9,10] These findings are at variance with the recent observation that two-thirds of patients with polycythemia vera display adequate suppression

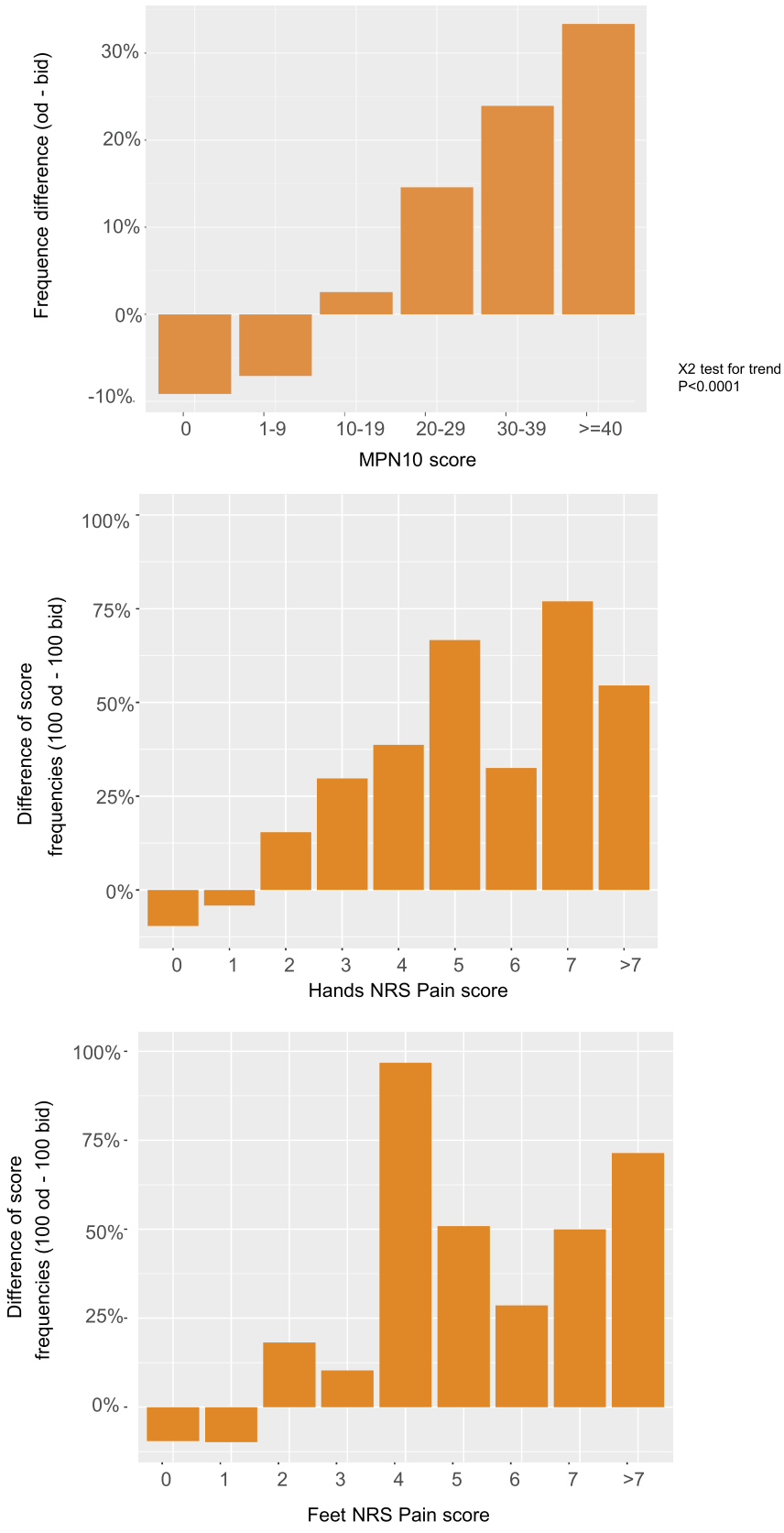



**FIGURE 3** Difference between MPN-10 score frequencies and pain scores for hands and feet according to the randomized aspirin regimen. In the upper panel, the MPN-10 score comprises the sum of 10 disease-specific symptoms, ranging from 0 (absence of any symptom) to 100 (all symptoms present at the worst possible degree). Bars represent the percent difference of MPN-10 scores between patients on the once-daily versus twice-daily regimen at each study visit. Moreover, patients were asked at each visit to circle a number between zero and 10 that best represented the pain intensity on their hands (middle panel) and feet (lower panel) over the week preceding the study visit, zero representing "no pain at all" and 10 representing "the worst pain ever possible" (numerical rating scale [NRS]). Bars represent the difference of each score frequency between patients on the aspirin once-daily regimen and patients on the twice-daily regimen.

of serum $TXB_2$ in response to a once-daily 100-mg aspirin regimen,[7] thus questioning the validity of extrapolating clinical trial results of patients with polycythemia vera[6] to patients with ET.

The present study assessed the long-term pharmacodynamic efficacy of and adherence with a low-dose aspirin twice-daily regimen, as well as its safety and tolerability, as compared with the standard once-

daily regimen in a relatively large cohort of aspirin-treated ET patients. Our findings demonstrate the feasibility of restoring an adequate aspirin response and maintaining it over an extended follow-up, with no evidence of reduced compliance or tolerability and no novel safety signals associated with more frequent dosing. The improved suppression of platelet COX-1 activity, assessed ex vivo, was associated with significantly reduced $TXA_2/TXB_2$ biosynthesis in vivo, as reflected by a substantial decrease in urinary 11-dehydro-$TXB_2$ excretion. The baseline excretion rate of this urinary $TXA_2/TXB_2$ metabolite was recently reported to be associated with all-cause and cardiovascular mortality, irrespective of aspirin use, in 3044 participants in the Framingham Heart Study over a 12-year follow-up,[41] as well as with serious vascular events in the placebo-controlled ASCEND trial in patients with diabetes and no previous cardiovascular disease.[42] Therefore, the finding of reduced in vivo $TXA_2$ biosynthesis in patients with ET suggests that the optimized aspirin regimen may effectively prevent $TXA_2$-dependent vascular events in this clinical setting.

A disease-related, clinical manifestation of ET is represented by microvascular disturbances (erythromelalgia, headache, visual disturbances, dizziness, tinnitus), which occur in up to one third of ET patients and impair their quality of life.[43] The high percentage of microvascular symptoms was recently confirmed by two cohort studies of 1000 ET patients each, with a prevalence of 29%.[44,45] Microvascular disorders have been reported to be predictive of the risk of recurrent thrombotic events after TIA[46] or venous thrombosis.[47]

According to the 2013 ELN criteria, the improvement in disease-specific symptoms, as listed in the MPN-10, should be considered when assessing the therapeutic response in ET and PV patients.[48] Cytoreductive treatment has a favorable impact in reducing high symptom burden,[49] but this therapeutic strategy is not recommended in low-risk patients. Previous non-randomized studies suggested that aspirin might reduce microvascular symptoms.[50,51] Moreover, the use of aspirin twice daily has been proposed by expert consensus[2] and NCCN guidelines[52] to reduce microvascular symptoms, although the quality of the evidence supporting this proposal is limited and relies on small, non-randomized studies.

In this study, we provide evidence that low-dose aspirin taken twice daily can significantly and consistently reduce the overall symptom burden, as compared to once-daily dosing, especially in patients with severe symptoms and erythromelalgia. The proportion of patients receiving cytoreductive treatment was comparable in both arms (Table 1), thus a confounding effect of cytoreduction on the reduction of symptoms seems unlikely. Therefore, the optimized regimen of aspirin given twice- rather than once-daily may represent an effective strategy for controlling the symptom burden in patients with ET.

Our finding that improved platelet inhibition by twice-daily aspirin was associated with persistently lower frequency of severe peripheral microvascular pain deserves further investigation, but is consistent with the involvement of $TXA_2$-mediated platelet activation and vasoconstriction in these microvascular disturbances.[50,51] The benefits of the twice-daily aspirin regimen included not only microvascular symptoms, but also a reduction of the whole symptom burden. Further studies are needed to understand and unravel underlying mechanisms.

The present study has several limitations. First, its sample size was not powered to assess hard clinical efficacy and safety outcomes, and their low numbers preclude any unequivocal interpretation. Second, the requirement that patients be already on low-dose aspirin therapy for inclusion in the study limits extrapolation to newly diagnosed, untreated ET patients. However, the relative ease of performing serum $TXB_2$ measurements before and after low-dose aspirin administration allows determining the response pattern in newly diagnosed patients and assessing the need for a twice-daily regimen. Third, the lack of measurement of agonist-induced platelet aggregation precludes any comparison with the pharmacodynamic efficacy of other antiplatelet agents that might be considered as an alternative or in addition to aspirin in ET patients. However, the effect of aspirin is variably detected by functional assays, potentially leading to misclassification of a normal aspirin response as aspirin "resistance", owing to poor intra-subject reproducibility of platelet aggregation inhibition upon repeated measurements.[36] Finally, the open-label design may limit the interpretation of the unblinded assessment of clinical symptoms and outcomes.

## 5 | CONCLUSIONS

Despite these limitations, we believe our findings have clinical implications for the antithrombotic treatment of patients with ET. These include the availability of a more effective aspirin regimen for precision antiplatelet therapy in the individual patient with ET. Moreover, they provide a rationale for revising current clinical practice guidelines for antiplatelet therapy in ET patients.

In this trial, twice-daily low-dose aspirin was consistently and persistently superior to the standard once-daily regimen in suppressing platelet $TXA_2$ production in patients with ET, and reduced microvascular symptoms with no detectable excess of bleeding complications and gastrointestinal discomfort.

### AUTHOR CONTRIBUTIONS

AT, BR, CP, and VDS conceived and designed the ARES trial and drafted the manuscript; DS and FR coordinated data collection; GP and BR measured urine and serum biomarkers; AT performed independent data analyses. All other Authors recruited, randomized and followed patients, collected biological samples and performed pre-analytical procedures throughout the study. All the Authors critically reviewed the manuscript, decided to submit it for publication, and vouch for the completeness and accuracy of the data and analyses as well as the fidelity of the study to the protocol. Complete list of the members of the Aspirin Regimens in Essential Thrombocythemia (ARES) Investigators appears in "Appendix."

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## CONFLICT OF INTEREST STATEMENT

AI: advisory board for: AOP Health, Bristol Myers Squibb, Incyte, Novartis; speaker fees from Bristol Myers Squibb, Glaxo Smith Kline, Incyte, Novartis, Pfizer. AMV: advisory board for: AOP Health, Blueprint, Bristol Myers Squibb, Glaxo Smith Kline, Incyte; speaker fees from AbbVie, AOP Health, Blueprint, Brisol Myers Squibb, Glaxo Smith Kline, Incyte, Jazz, Novartis. AR: advisory board for: Alexion, Bristol Myers Squibb, Grifols, Novartis, SOBI. AT: speaker fees from Bayer, Novo-Nordisk, Roche, Sanofi, Stago, Werfen. BR: consultant fee from Aboca. CB: speaker fees from Incyte, Novartis, Pfizer. CP: consultant and speakers fees from AbbVie, Eli Lilly, and Tremeau; Past-Chair of the Scientific Advisory Board of the International Aspirin Foundation; grants to the Institution for investigator-initiated research from Bayer and Cancer Research UK. DC: advisory board for AOP Health; speaker fees from Bristol Myers Squibb, Glaxo Smith Kline, Incyte, Novartis, Pfizer. EB: advisory board for: SOBI; speaker fees from Alexion, Incyte, Novartis. EE: speaker fees from AbbVie, Novartis. ER: speaker fees from Amgen, Bristol Myers Squibb, Grifols, Janssen, Novartis, SOBI. FP: advisory board for: AbbVie, AOP Health, Bristol Myers Squibb, CTI Biopharma, Glaxo Smith Kline, Kartos-Telios, Novartis; speaker fees from AbbVie, AOP Health, Bristol Myers Squibb, Grifols, Glaxo Smith Kline, Novartis, SOBI. FR: advisory board for: Argenx, Amgen, Grifols, Novartis. GC: advisory board for: Amgen, AOP Health, Grifols, Novartis, Novo Nordisk, SOBI, Takeda; speaker fees from AOP Health, Bristol Myers Squibb, Grifols, Novartis. GGL: advisory board for: Novartis; speaker fees from Novartis. IB: speaker fees from Novartis. NV: advisory board for: Amgen, Grifols, Novartis, Sanofi, SOBI. VDS: advisory board for: AOP Health, Argenx, Bristol Myers Squibb, Glaxo Smith Kline, Grifols, Novartis, SOBI, Takeda; speaker fees from Abbvie, Alexion, Amgen, Bristol Myers Squibb, Grifols, Leo Pharma, Novartis, Novo Nordisk, Sanofi, Takeda; research grant from Alexion. The other authors have no conflicts of interest to disclose.

## DATA AVAILABILITY STATEMENT

Deidentified individual participant data that underlie the reported results will be made available 3 months after publication for a period of 3 years after the publication date. The study dataset is available at www.osf.io, upon request. Requests for access should be sent to Alberto Tosetto (alberto.tosetto@aulss8.veneto.it); they will be subjected to review and approval by the Steering Committee of the study.

## ORCID

*Alberto Tosetto* https://orcid.org/0000-0002-0119-5204
*Alessandra Iurlo* https://orcid.org/0000-0002-4401-0812
*Marco Ruggeri* https://orcid.org/0000-0003-1557-0957
*Elena Maria Elli* https://orcid.org/0000-0001-5576-5412
*Maria Luigia Randi* https://orcid.org/0000-0001-7945-1864
*Irene Bertozzi* https://orcid.org/0000-0003-2888-9596
*Giuseppe Gaetano Loscocco* https://orcid.org/0000-0002-6241-1206
*Alessandro M. Vannucchi* https://orcid.org/0000-0001-5755-0730
*Valerio De Stefano* https://orcid.org/0000-0002-5178-5827

## REFERENCES

1. Tefferi A, Pardanani A. Essential Thrombocythemia. *N Engl J Med*. 2019;381(22):2135-2144.
2. Tefferi A, Vannucchi AM, Barbui T. Essential thrombocythemia: 2024 update on diagnosis, risk stratification, and management. *Am J Hematol*. 2024;99(4):697-718.
3. https://www.nccn.org/professionals/physician_gls/pdf/mpn.pdf.
4. Alvarez-Larran A, Pereira A, Guglielmelli P, et al. Antiplatelet therapy versus observation in low-risk essential thrombocythemia with a CALR mutation. *Haematologica*. 2016;101(8):926-931.
5. Alvarez-Larran A, Cervantes F, Pereira A, et al. Observation versus antiplatelet therapy as primary prophylaxis for thrombosis in low-risk essential thrombocythemia. *Blood*. 2010;116(8):1205-1210.
6. Landolfi R, Marchioli R, Kutti J, et al. Efficacy and safety of low-dose aspirin in polycythemia vera. *N Engl J Med*. 2004;350(2):114-124.
7. Petrucci G, Giaretta A, Ranalli P, et al. Platelet thromboxane inhibition by low-dose aspirin in polycythemia vera: ex vivo and in vivo measurements and in silico simulation. *Clin Transl Sci*. 2022;15:2958-2970.
8. Gruppo Italiano Studio Policitemia. Low-dose aspirin in polycythaemia vera: a pilot study. *Br J Haematol*. 1997;97(2):453-456.
9. Pascale S, Petrucci G, Dragani A, et al. Aspirin-insensitive thromboxane biosynthesis in essential thrombocythemia is explained by accelerated renewal of the drug target. *Blood*. 2012;119(15):3595-3603.
10. Dragani A, Pascale S, Recchiuti A, et al. The contribution of cyclooxygenase-1 and -2 to persistent thromboxane biosynthesis in aspirin-treated essential thrombocythemia: implications for antiplatelet therapy. *Blood*. 2009;115(5):1054-1061.
11. De Stefano V, Rocca B, Tosetto A, et al. The Aspirin Regimens in Essential Thrombocythemia (ARES) phase II randomized trial design: implementation of the serum thromboxane B2 assay as an evaluation tool of different aspirin dosing regimens in the clinical setting. *Blood Cancer J*. 2018;8(6):49.
12. Rocca B, Tosetto A, Betti S, et al. A randomized double-blind trial of 3 aspirin regimens to optimize antiplatelet therapy in essential thrombocythemia. *Blood*. 2020;136(2):171-182.
13. Badimon L, Vilahur G, Rocca B, Patrono C. The key contribution of platelet and vascular arachidonic acid metabolism to the pathophysiology of atherothrombosis. *Cardiovasc Res*. 2021;117(9):2001-2015.
14. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391-2405.
15. Finazzi G, Carobbio A, Thiele J, et al. Incidence and risk factors for bleeding in 1104 patients with essential thrombocythemia or prefibrotic myelofibrosis diagnosed according to the 2008 WHO criteria. *Leukemia*. 2012;26(4):716-719.
16. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-381.
17. Catella-Lawson F, Reilly MP, Kapoor SC, et al. Cyclooxygenase inhibitors and the antiplatelet effects of aspirin. *N Engl J Med*. 2001;345(25):1809-1817.
18. Patrono C, Ciabattoni G, Pinca E, et al. Low dose aspirin and inhibition of thromboxane B2 production in healthy subjects. *Thromb Res*. 1980;17(3–4):317-327.
19. Ciabattoni G, Maclouf J, Catella F, FitzGerald GA, Patrono C. Radioimmunoassay of 11-dehydrothromboxane B2 in human plasma and urine. *Biochim Biophys Acta*. 1987;918(3):293-297.
20. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003340.pdf.

21. Scherber R, Dueck AC, Johansson P, et al. The Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF): international prospective validation and reliability trial in 402 patients. *Blood*. 2011; 118(2):401-408.
22. Emanuel RM, Dueck AC, Geyer HL, et al. Myeloproliferative neoplasm (MPN) symptom assessment form total symptom score: prospective international assessment of an abbreviated symptom burden scoring system among patients with MPNs. *J Clin Oncol*. 2012;30(33):4098-4103.
23. Mesa R, Miller CB, Thyne M, et al. Myeloproliferative neoplasms (MPNs) have a significant impact on patients' overall health and productivity: the MPN landmark survey. *BMC Cancer*. 2016;16:167.
24. Hjermstad MJ, Fayers PM, Haugen DF, et al. Studies comparing numerical rating scales, verbal rating scales, and visual analogue scales for assessment of pain intensity in adults: a systematic literature review. *J Pain Symptom Manag*. 2011;41(6):1073-1093.
25. Rabeneck L, Wristers K, Goldstein JL, Eisen G, Dedhiya SD, Burke TA. Reliability, validity, and responsiveness of severity of dyspepsia assessment (SODA) in a randomized clinical trial of a COX-2-specific inhibitor and traditional NSAID therapy. *Am J Gastroenterol*. 2002;97(1):32-39.
26. Schulman S, Kearon C, Subcommittee on Control of Anticoagulation of the S, Standardization Committee of the International Society on T, Haemostasis. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. *J Thromb Haemost*. 2005;3(4):692-694.
27. Kaatz S, Ahmad D, Spyropoulos AC, Schulman S, Subcommittee on Control of A. Definition of clinically relevant non-major bleeding in studies of anticoagulants in atrial fibrillation and venous thromboembolic disease in non-surgical patients: communication from the SSC of the ISTH. *J Thromb Haemost*. 2015;13(11):2119-2126.
28. Lex A, Gehlenborg N, Strobelt H, Vuillemot R, Pfister H. UpSet: visualization of intersecting sets. *IEEE Trans Vis Comput Graph*. 2014; 20(12):1983-1992.
29. Barbui T, Tefferi A, Vannucchi AM, et al. Philadelphia chromosome-negative classical myeloproliferative neoplasms: revised management recommendations from European LeukemiaNet. *Leukemia*. 2018; 32(5):1057-1069.
30. Patrono C, Rocca B. Measurement of thromboxane biosynthesis in health and disease. *Front Pharmacol*. 2019;10:1244.
31. Hultcrantz M, Bjorkholm M, Dickman PW, et al. Risk for arterial and venous thrombosis in patients with myeloproliferative neoplasms: a population-based cohort study. *Ann Intern Med*. 2018;168(5):317-325.
32. Chu DK, Hillis CM, Leong DP, Anand SS, Siegal DM. Benefits and risks of antithrombotic therapy in essential Thrombocythemia: a systematic review. *Ann Intern Med*. 2017;167(3):170-180.
33. Born G, Patrono C. Antiplatelet drugs. *Br J Pharmacol*. 2006;147-(Suppl 1):S241-S251.
34. Patrono C. Aspirin as an antiplatelet drug. *N Engl J Med*. 1994; 330(18):1287-1294.
35. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/203697_aspirin_toc.cfm.
36. Santilli F, Rocca B, De Cristofaro R, et al. Platelet cyclooxygenase inhibition by low-dose aspirin is not reflected consistently by platelet function assays: implications for aspirin "resistance". *J Am Coll Cardiol*. 2009;53(8):667-677.
37. Reilly IA, FitzGerald GA. Inhibition of thromboxane formation in vivo and ex vivo: implications for therapy with platelet inhibitory drugs. *Blood*. 1987;69(1):180-186.
38. Patrono C, Ciabattoni G, Patrignani P, et al. Clinical pharmacology of platelet cyclooxygenase inhibition. *Circulation*. 1985;72(6):1177-1184.
39. Bhala N, Emberson J, Merhi A, et al. Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: meta-analyses of individual participant data from randomised trials. *Lancet*. 2013; 382(9894):769-779.
40. Patrono C, Garcia Rodriguez LA, Landolfi R, Baigent C. Low-dose aspirin for the prevention of atherothrombosis. *N Engl J Med*. 2005; 353(22):2373-2383.
41. Rade JJ, Barton BA, Vasan RS, et al. Association of thromboxane generation with survival in aspirin users and nonusers. *J Am Coll Cardiol*. 2022;80(3):233-250.
42. Petrucci G, Buck GA, Rocca B, et al. Thromboxane biosynthesis and future events in diabetes: the ASCEND trial. *Eur Heart J*. 2024;45(15): 1355-1367.
43. Wolanskyj AP, Schwager SM, McClure RF, Larson DR, Tefferi A. Essential thrombocythemia beyond the first decade: life expectancy, long-term complication rates, and prognostic factors. *Mayo Clin Proc*. 2006;81(2):159-166.
44. Loscocco GG, Gesullo F, Capecchi G, et al. One thousand patients with essential thrombocythemia: the Florence-CRIMM experience. *Blood Cancer J*. 2024;14(1):10.
45. Gangat N, Karrar O, Al-Kali A, et al. One thousand patients with essential thrombocythemia: the Mayo Clinic experience. *Blood Cancer J*. 2024;14(1):11.
46. De Stefano V, Carobbio A, Di Lazzaro V, et al. Benefit-risk profile of cytoreductive drugs along with antiplatelet and antithrombotic therapy after transient ischemic attack or ischemic stroke in myeloproliferative neoplasms. *Blood Cancer J*. 2018; 8(3):25.
47. De Stefano V, Ruggeri M, Cervantes F, et al. High rate of recurrent venous thromboembolism in patients with myeloproliferative neoplasms and effect of prophylaxis with vitamin K antagonists. *Leukemia*. 2016;30(10):2032-2038.
48. Barosi G, Mesa R, Finazzi G, et al. Revised response criteria for polycythemia vera and essential thrombocythemia: an ELN and IWG-MRT consensus project. *Blood*. 2013;121(23):4778-4781.
49. Mazza GL, Mead-Harvey C, Mascarenhas J, et al. Symptom burden and quality of life in patients with high-risk essential thrombocythaemia and polycythaemia vera receiving hydroxyurea or pegylated interferon alfa-2a: a post-hoc analysis of the MPN-RC 111 and 112 trials. *Lancet Haematol*. 2022;9(1):e38-e48.
50. Patrono C, Rocca B, De Stefano V. Platelet activation and inhibition in polycythemia vera and essential thrombocythemia. *Blood*. 2013; 121(10):1701-1711.
51. Michiels JJ, Berneman Z, Schroyens W, et al. Platelet-mediated erythromelalgic, cerebral, ocular and coronary microvascular ischemic and thrombotic manifestations in patients with essential thrombocythemia and polycythemia vera: a distinct aspirin-responsive and coumadin-resistant arterial thrombophilia. *Platelets*. 2006;17(8): 528-544.
52. Mesa RA, Jamieson C, Bhatia R, et al. NCCN guidelines insights: myeloproliferative neoplasms, version 2.2018. *J Natl Compr Cancer Netw*. 2017;15(10):1193-1207.
53. Barbui T, Finazzi G, Carobbio A, et al. Development and validation of an International Prognostic Score of thrombosis in World Health Organization-essential thrombocythemia (IPSET-thrombosis). *Blood*. 2012;120(26):5128-5133.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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## APPENDIX A: STUDY GROUP MEMBERS

The members of the Aspirin Regimens in Essential Thrombocythemia (ARES) trial are:

Valerio De Stefano, Elena Rossi, Silvia Betti, Tommaso Za, Angela Ciminello, Francesca Bartolomei, Denise Soldati, Giulia De Santis (Catholic University School of Medicine, and Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Italy);

Carlo Patrono, Bianca Rocca, Giovanna Petrucci, Duaa Hatem (Catholic University School of Medicine, Roma, Italy);

Marco Ruggeri, Alberto Tosetto, Giuseppe Carli, Ilaria Nichele, Stefania Bellesso (Ospedale San Bortolo, Vicenza, Italy);

Francesco Rodeghiero, Andrea Timillero (Hematology Project Foundation, Vicenza, Italy);

Alessandra Iurlo, Daniele Cattaneo, Cristina Bucelli, Silvia Artuso (Foundation IRCCS Ca′ Granda Ospedale Maggiore Policlinico, and University of Milan, Milano, Italy);

Alfredo Dragani, Mauro Di Ianni, Paola Ranalli, Giovanna Summa, Celeste Santone (Santo Spirito Hospital, Pescara, and G. D'Annunzio University, Chieti, Italy);

Francesca Palandri, Nicola Vianelli, Giuseppe Auteri (IRCCS Sant'Orsola-Malpighi Hospital, Bologna, Italy);

Eloise Beggiato, Giuseppe Lanzarone, Miriana Arminio (AOU Città della Salute e della Scienza, Torino, Italy);

Elena Maria Elli, Stefania Priolo, Nora Giello, Rossella Renso (Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy);

Maria Luigia Randi, Irene Bertozzi, Giulia Bogoni (University of Padova, Padova, Italy);

Alessandro Maria Vannucchi, Paola Guglielmelli, Giuseppe Gaetano Loscocco, Francesco Mannelli, Giacomo Coltro, Chiara Paoli, Enrica Ravenda (CRIMM-Center of Research and Innovation of Myeloproliferative Neoplasms, Azienda Ospedaliera Universitaria Careggi and University of Firenze, Firenze, Italy);

Giorgina Specchia, Francesco Albano, Alessandra Ricco, Paola Carluccio, Marianna Gentile (University of Bari, Bari, Italy).