REVIEW ARTICLE

Aspirin in essential thrombocythemia. For whom? What formulation? What regimen?

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Abstract

Essential thrombocythemia (ET) is a *BCR-ABL1*-negative myeloproliferative neoplasm, the most common clinical manifestations of which include arterial and venous thrombosis, bleeding and vasomotor/microvascular disturbances. Low-dose (81-100 mg) aspirin once daily, which irreversibly inhibits platelet thromboxane A2 (TxA2) production by acetylating cyclo-oxygenase-1, is the recommended treatment for the control of vascular events in all ET risk categories, except patients at very low risk, who need aspirin for treatment of vasomotor/microvascular disturbances only. Simple observation should be preferred over aspirin prophylaxis in low-risk patients with platelet counts >1,000x10^9/L or harboring *CALR* mutations. Plain aspirin should be preferred over enteric coated aspirin because some ET patients display poor responsiveness ("resistance") to the latter. When treated with a once daily aspirin regimen, adequate inhibition of platelet TxA2 production (measured as serum thromboxane B2 level) does not persist for 24 h in most patients. This phenomenon is associated with the patients' platelet count and the number (but not the fraction) of circulating immature reticulated platelets with non-acetylated cyclo-oxygenase-1 and is therefore consequent to high platelet production (the hallmark of ET), rather than increased platelet turnover (which is normal in ET). Twice daily aspirin administration overcame this problem and proved safe in small studies. Although additional data on gastrointestinal tolerability will be useful, the twice daily regimen could already be implemented in clinical practice, considering its favorable risk/benefit profile. However, patients whose platelet count has been normalized could still be treated with the once daily regimen, because they would otherwise be unnecessarily exposed to a potential small risk of gastrointestinal discomfort.

Essential thrombocythemia in the realm of myeloproliferative neoplasms

Essential thrombocythemia (ET) belongs to the group of *BCR-ABL1*-negative myeloproliferative neoplasms, which also includes polycythemia vera and primary myelofibrosis. Myeloproliferative neoplasms are hematopoietic stem-cell disorders that are characterized by the presence of mutually exclusive driver mutations of the downstream kinase *JAK2* (Janus kinase 2), the endoplasmic reticulum chaperone *CALR* (calreticulin) or the thrombopoietin receptor *MPL* (myeloproliferative leukemia virus oncogene), which are associated with constitutive activation of hematopoietic pathways. In ET, the most frequent mutation is *JAK2*^{V617F} (present in about 55% of cases), followed by *CALR* mutations (about 20%) and *MPL* mutations (about 4%); no known mutation is detected in about 20% of ET patients.[1] Isolated thrombocytosis may be the first manifestation of polycythemia vera or primary myelofibrosis. It is indispensable to distinguish ET from pre-fibrotic primary myelofibrosis or other myeloid neoplasms, mostly by means of morphological examination of the bone marrow.[1,2] Rare cases have been described of hereditary, familial thrombocythemia associated with germline mutations of *JAK2*, *MPL* or the thrombopoietin gene (*THPO*).[3] Major criteria for a diagnosis of ET are: (i) platelet count >450x10^9/L, (ii) bone marrow megakaryocyte proliferation and loose clusters, (iii) not meeting World Health Organization (WHO) criteria for other myeloid neoplasms, and (iv) mutated *JAK2/CALR/MPL*. Minor criteria include the presence of other clonal markers and no evidence of reactive thrombocytosis. All four major or three major plus one minor criteria are required to make the diagnosis.[4]

The annual incidence of ET is estimated to be between

1.2 and 3.0 cases per 100,000,[5,6] while the estimated prevalence in Western countries is 24-30 cases per 100,000.[7,8] The clinical picture is highly variable, with most patients being asymptomatic at presentation. Signs and symptoms include microcirculatory and vasomotor manifestations, such as vascular headaches, dizziness, vertigo, visual disturbances, acral dysesthesia, acrocyanosis and erythromelalgia.[5,9] The most common severe clinical manifestations include arterial and, more rarely, venous thromboembolic events and bleeding manifestations.[5,9,10] The clinical course is usually mild, with a median survival that is not very different from normal.[5,7] The 10-year incidence of transition to acute myeloid leukemia or post-ET myelofibrosis is rare (<1%).[10] The main goal of treatment of ET is to alleviate the microcirculatory and vasomotor signs and symptoms and reduce the risk of thrombotic and hemorrhagic complications. Aspirin is the recommended treatment for the control of vascular events in most ET patients, although its use is still a matter of debate.[11,12]

Thrombosis and bleeding in essential thrombocythemia

Incidence and prevalence

Thrombosis and bleeding are among the initial manifestations of ET: a meta-analysis of published studies up to August 2018 revealed that, among 6,610 patients, the prevalence of thrombotic and bleeding events at diagnosis was 20.7% (95% confidence interval [95% CI]: 16.6-25.5) and 7.3% (95% CI: 5.3-10.0), respectively.[13] In an international collaborative study of 891 patients, 109 (12%) experienced arterial (n=79) or venous (n=37) thrombosis after a median follow-up of 6.2 years.[14] Venous thrombotic events may involve atypical vascular districts, such as cerebral sinuses and splanchnic veins.[10] An analysis of studies published in the previous 15 years showed that the median incidences of bleeding and major bleeding events were 2.2% and 0.79% patient-years, respectively.[15] Most bleeds were gastrointestinal, while the most frequent fatal bleeding was intracerebral hemorrhage.[15]

The occurrence of both thrombotic and bleeding events in ET highlights an apparently paradoxical coexistence of opposite clinical manifestations of abnormalities of hemostasis, which stimulated several investigations of hemostasis parameters in these patients.

Hemostasis parameters in essential thrombocythemia

Global tests of primary hemostasis

As in congenital hemostatic defects,[16] global tests of primary hemostasis display different sensitivities to the acquired defects of ET patients: prolongation of the bleeding time is observed in a minority of patients,[17-21] while prolonged closure times of both PFA-100 cartridges are more frequent.[22] Prolongation of the bleeding time after the oral administration of aspirin is more pronounced in ET patients than in healthy subjects,[23,24] suggesting that aspirin can unmask underlying defects of primary hemostasis.

Platelet thromboxane A2 production

Some studies have found high total serum levels of thromboxane B2 (TxB2), a stable metabolite of thromboxane A2 (TxA2) in ET patients;[25-27] however, these high levels were most likely a reflection of thrombocytosis, because when results were expressed relative to the platelet count, they were comparable to normal.[25,27]

Platelet aggregation in vitro

Some studies showed that high percentages of ET patients display spontaneous platelet aggregation,[19,20,28,29] which is associated with increased risk of vascular events in the general population.[30,31] Studies of *in vitro* agonist-induced platelet aggregation revealed a high degree of inter-individual variability and contrasting results.[32] In general, results obtained by the traditional light transmission aggregometry (LTA) technique in citrate-anticoagulated platelet-rich plasma whose platelet count had been normalized to pre-defined standardized values by the addition of autologous plasma usually documented defects of agonist-induced platelet aggregation.[18,19,21-23,32-34] However, the normalization of platelet count in platelet-rich plasma by autologous plasma is now contraindicated,[35] because LTA results are not affected by the sample platelet count[36,3] and dilution of platelet-rich plasma by autologous plasma inhibits platelet aggregation.[33,36] In contrast to LTA studies, experiments performed in whole blood by multiple electrode aggregometry showed normal or even increased platelet aggregation,[28,29,32,34,38,39] consistent with the demonstration that there is a strong positive correlation between the sample platelet count and platelet aggregation measured by multiple electrode aggregometry.[37] A study in which platelet aggregation was tested in parallel in the same patients under five different experimental conditions confirmed that agonist-induced aggregation of ET platelets is normal when confounders do not influence the results.[34] The only agonist that consistently did not induce normal aggregation of ET platelets was epinephrine,[17-19,25,34,40] due to decreased expression of α_2-adrenergic receptors and/or abnormalities of the transduction pathway.[17,40] *JAK2*^{V617F} did not affect the *in vitro* aggregation response of ET platelets to agonists.[34,41]

Platelet granule content

ET platelets often display acquired storage pool deficiency, characterized by deficiency of the constituents of platelet granules.[17,23,25,34,42] Although the presence of an abnormal clone with defective δ-granules has been hypo-

thesized,[25] "exhaustion" of platelet granules by *in vivo* platelet activation and participation in microthrombi is the prevalent pathogenic mechanism.

Markers of in vivo activation of hemostasis

In vivo activation of hemostasis in ET is documented by the presence in peripheral blood of platelets expressing activation markers, platelet/leukocytes hetero-aggregates, high plasma levels of platelet granule constituents and, less frequently, markers of activation of coagulation.[32,34,43-45] The levels of urinary 11-dehydro-TxB2 were higher in 40 ET patients than in 26 healthy sex- and age-matched controls and were decreased by aspirin, suggesting that this urinary metabolite is largely derived from *in vivo* platelet activation.[46] *In vivo* platelet activation causes the release of procoagulant platelet-derived microparticles[47] and contributes to dysfunction of von Willebrand factor (VWF).[48]

Plasma von Willebrand factor

Acquired VWF deficiency with loss of VWF high molecular weight multimers was first described in seven patients,[49] associated with increased proteolysis[50] and high platelet count[51] and improved after reduction of platelet counts by chemotherapy.[49] These findings were later confirmed by other investigators.[10,52] ET patients who showed an excessive prolongation of bleeding time by aspirin had significantly decreased levels of large VWF multimers in plasma.[24]

Platelet survival and turnover

Platelet turnover increases under conditions in which decreased platelet survival caused by heightened peripheral platelet consumption is associated with increased compensatory platelet production. The most commonly used method to evaluate platelet turnover is based on the measurement of reticulated platelets, i.e., newly formed platelets retaining some RNA. An increased percentage of circulating reticulated platelets is suggestive of increased turnover, while an increased number but normal percentage is simply a reflection of high platelet count. The number of reticulated platelets was increased, but its percentage was normal in ET patients without vascular disorders (Table 1).[39,53,54] In contrast, ET patients with vascular disorders display decreased platelet survival[10,55] and an increased percentage of circulating reticulated platelets.[56,57] Aspirin treatment not only cured the signs and symptoms of erythromelalgia,[10] but also normalized platelet survival and the percentage of reticulated platelets.[10,56] Therefore, platelet turnover is normal in ET patients without vascular disorders, while an increased count with a normal percentage of reticulated platelets is simply a reflection of increased platelet production.

Potential mechanisms of bleeding and thrombosis in essential thrombocythemia

Platelets of ET patients interact with activated leukocytes,[42,45,47,58-60] to form thrombi, especially in the microcirculation. The release of procoagulant platelet-derived microparticles contributes to the dissemination of the thrombogenic stimuli. Platelets that deaggregate from these thrombi and return to the circulation are "exhausted", displaying acquired storage pool deficiency[17,23,25,34,42] and contributing to increase the bleeding risk together with acquired von Willebrand disease in patients with extremely high platelet counts. The thrombotic risk is increased by the presence of *JAK2*^{V617F},[62,63] which plays a role in neutrophil extracellular trap formation.[64] In contrast, the risk of thrombosis is low in patients with *CALR*-mutated ET, which affects relatively young individuals and is characterized by markedly elevated platelet count.[65]

Risk factors for bleeding and thrombosis in patients with essential thrombocythemia

Clinical risk factors for bleeding are not particularly useful to define the bleeding risk in ET patients. Duration of dis-

Table 1. Reticulated platelet count and reticulated platelet fraction in healthy subjects and patients with essential thrombocythemia.

Study	Reticulated platelet count, x10^9/L		Reticulated platelet fraction, %	
	Healthy subjects	ET patients	Healthy subjects	ET patients
Kienast *et al.*[53]	21.3±7.9 N=50	75.5 N=2	8.6±2.8 N=50	8.2 N=2
Rinder *et al.*[56]	6±6 N=83	36±14 N=5	3.4±1.3 N=83	4.2±1.9 N=5
Pedersen *et al.*[39]	6.9 (5.5-10.3) N=24	12.3 (9.8-18.7) N=24	2.6 (2.1-3.9) N=24	2.8 (2.3-3.4) N=24
Scavone *et al.*[54]	19.4 (17.1-25.2) N=8	34.3 (26.9-50.7) N=15	10.6 (7.7-12.8) N=8	9.1 (7.5-12.3) N=15

Results are expressed as mean±standard deviation or median (interquartile range), as in the original publications. The data refer to patients with essential thrombocythemia (ET) without thrombotic events. In the study by Rinder *et al.*, seven additional ET patients with thrombotic events had increased reticulated platelet count (94±33x10^9/L) and reticulated platelet fraction (13.8±5.1%),[56] suggesting that increased platelet turnover is associated with thrombosis and not with ET (see text).

ease, hypertension, bleeding history, splenomegaly and male sex were identified as bleeding risk factors in some studies.[15] In a retrospective analysis of 891 ET patients, previous hemorrhage and aspirin use were independently associated with bleeding risk.[66] The bleeding risk associated with aspirin use was found to be particularly relevant for patients harboring *CALR* mutations[67] or with platelet counts >1,000x10^9/L.[66,68] The combination of platelet count >1,000x10^9/L, leukocytosis and acquired von Willebrand disease was identified as a biological risk factor for bleeding.[15]

Risk stratification for thrombosis in ET patients has been mostly based on the presence of conventional risk factors, such as age >60 years and a positive history of thrombosis.[5] The revised International Prognostic Score for Thrombosis in ET (IPSET-thrombosis) model also considered the presence of a *JAK2* mutation and of additional cardiovascular risk factors to stratify ET patients into four categories of thrombosis risk:[69,70] (i) very low risk: age ≤60 years, absent *JAK2* mutation, no prior history of thrombosis; (ii) low risk: age ≤60 years, *JAK2* mutation, no prior history of thrombosis; (iii) intermediate risk: age >60 years, absent *JAK2* mutation, no prior history of thrombosis; and (iv) high risk: history of thrombosis at any age or age >60 years with *JAK2* mutation (Table 2). The revised IPSET-thrombosis model was validated in 1,381 patients and found to be a better fit than the earlier IPSET-thrombosis score.[71] A recent retrospective real-world analysis of Medicare patients (aged ≥65 years) newly diagnosed with intermediate/high-risk ET revealed that the mortality risk during a 25.5-month follow-up was significantly higher among patients who experienced a thrombotic event.[72]

Prevention of thrombosis in essential thrombocythemia patients

Prevention of recurrent thrombosis in ET patients must follow the general guidelines for secondary prevention in the general population, including antiplatelet agents and anticoagulant drugs, depending on the clinical setting. Primary prevention of thrombosis is based on the prophylactic use of low-dose aspirin (81-100 mg daily) and cytoreduction. Aspirin is recommended for all risk categories, with the exception of patients at very low risk, who should only be given aspirin for treatment of vasomotor/microvascular disturbances;[70] cytoreduction with hydroxyurea or, alternatively, with peginterferon α2a or anagrelide is usually restricted to high-risk patients (Table 2).[70] Cytoreductive treatment should, however, also be considered in other risk categories, depending on the presence of additional conditions, such as von Willebrand disease, extreme thrombocytosis and/or leukocytosis, splenomegaly, vasomotor/microvascular disturbances or other disease-related symptoms not responsive to aspirin. The efficacy of aspirin in controlling vasomotor/microvascular disturbances is well recognized, despite the lack of controlled trials, thanks to the dramatic improvement of patients' signs and symptoms in response to aspirin treatment.[10,52,73,74] One study elegantly documented the tem-

Table 2. National Comprehensive Cancer Network (NCCN) Guidelines (version 3.2022) for risk stratification and management of patients with essential thrombocythemia.

Risk category	Patients' characteristics	Rate of thrombosis		Management
		Without CV risk factors	With CV risk factors	
Very low	Age ≤60 years No *JAK2* mutation No history of thrombosis	0.44%/year	1.05%/year	Manage CV risk factors No aspirin (aspirin 81-100 mg for patients with vasomotor/microvascular disturbances)
Low	Age ≤60 years *JAK2* mutation No history of thrombosis	1.59%/year	2.57%/year	Manage CV risk factors Aspirin (81-100 mg)
Intermediate	Age >60 years No *JAK2* mutation No history of thrombosis	1.44%/year	1.64%/year	Manage CV risk factors Aspirin (81-100 mg)
High	Any age + History of thrombosis Age >60 years + *JAK2* mutation	2.36%/year	4.17%/year	Manage CV risk factors Aspirin (81-100 mg) Cytoreductive therapy: *Preferred:* Hydroxyurea *Other recommended regimens:* Peginterferon α2a Anagrelide

CV: cardiovascular.

poral association between increased urinary levels of TxA2 metabolites and the development of erythromelalgia, which were both dramatically inhibited by the administration of low-dose aspirin.[75]

The efficacy of aspirin in primary prevention of major thrombotic events in ET has not been documented by randomized controlled trials. The potential benefit of aspirin is inferred from analogies with its use in polycythemia vera, in which low-dose aspirin has been shown to cause an approximately 60% reduction in the risk of the combined endpoint of nonfatal myocardial infarction, nonfatal stroke or death from cardiovascular causes and of venous thromboembolism, without increasing the risk of bleeding complications.[76] The indication for the use of aspirin also comes from observational, retrospective studies. Alvarez-Larrán *et al*. showed that, among ET patients <60 years of age and without a positive history of thrombosis, aspirin reduced the incidence of venous thromboembolism in carriers of *JAK2*^{V617F} and of arterial thrombosis in patients with cardiovascular risk factors, while it was ineffective in the remaining patients and increased the incidence of bleeding in those with a platelet count >1,000x10^9/L.[77] Another study on the same type of ET patients confirmed the safety and protective effect of aspirin against thrombosis in *JAK2*^{V617F} carriers, but not in *CALR* mutation carriers, in whom aspirin was not protective and increased the incidence of bleeding.[67] A recent consensus of experts on the management of *CALR*-mutated ET recommends a pure observational approach over aspirin prophylaxis in asymptomatic low-risk ET patients without cardiovascular risk factors, while cytoreduction should be preferred over aspirin for low-risk symptomatic patients with a platelet count of 1,000-1,500x10^9/L.[78] An observational study including high-risk ET patients >60 years old showed that the combination of aspirin plus cytoreductive therapy was superior to cytoreductive therapy alone in the primary prevention of thrombosis.[79]

Overall, protection from thrombosis by aspirin in ET appeared modest in a systematic review of 24 observational studies including 6,153 ET patients, which showed an estimated 26% reduction of thrombotic events from antiplatelet therapy (aspirin in 80% of patients),[80] lower than that observed in polycythemia vera.[76] Although this observation may also have alternative interpretations, it raised the question of whether the inhibition of TxA2 biosynthesis by aspirin is inadequate in all ET patients, because inadequate inhibition of TxA2 biosynthesis by aspirin is associated with insufficient antithrombotic efficacy.[81-83]

Aspirin as an antiplatelet and antithrombotic drug

Aspirin acetylates a serine residue at position 529 of cyclo-oxygenase-1 (COX-1), thus irreversibly inhibiting its metabolic pathway, which is responsible for the production of the platelet agonist and vasoconstrictive molecule TxA2.[83] Virtually complete inhibition of TxA2 synthesis throughout the 24-hour interval between doses is necessary to prevent thrombotic events.[83] Aspirin is widely used as an antithrombotic drug for the treatment of acute coronary syndromes and cerebrovascular accidents and for their secondary prevention.[83] Although the net clinical benefit of aspirin in primary prevention of coronary and cerebrovascular disorders in the general population is unclear,[83,84] it is well established in patients with polycythemia vera.[76] The very good antithrombotic efficacy of aspirin, despite its very selective pharmacodynamics, is explained by the fact that TxA2 contributes to the amplification of platelet activation by almost any platelet agonist and is essential for the full aggregation response of platelets.[85]

Inter-individual variability of pharmacological response to aspirin ("aspirin resistance")

At the beginning of the 21st century, several studies documented a high prevalence of poor inhibition of platelet function by aspirin (defined in some reports as "aspirin resistance") in treated patients.[86] However, a careful analysis of the published studies revealed flaws in the evaluation of the pharmacological response to aspirin, which was studied by LTA or other non-specific tests of platelet function, such as the PFA-100.[86,87] LTA is sensitive to several variables and should be performed only in specialized laboratories by dedicated personnel, following standardized procedures.[35,85] Although TxA2 contributes to the final extent of platelet aggregation,[85] aspirin cannot inhibit the initial response to the platelet agonists that are used in LTA studies which, therefore, lack the necessary specificity for the inhibitory effects of the drug.[85,86] Even when arachidonic acid, the direct precursor of TxA2, is used as a platelet agonist, the results obtained with this technique may overestimate the prevalence of poor responders to aspirin.[85] Methods that measure the capacity of platelets to synthesize TxA2 directly are preferable. Of these, measurement of the urinary levels of the TxB2 metabolite is not highly specific for platelet COX-1, because about 30% of it (or more in pathological conditions) derives from extra-platelet sources.[81] In contrast, serum TxB2 reflects the total capacity of platelets to synthesize TxA2 and is highly specific, because the contribution of other blood cells to its synthesis is marginal.[83,85] When the response to aspirin was correctly tested by measuring serum TxB2 levels, the frequency of poor responders was very low, suggesting that monitoring aspirin treatment with laboratory tests should not be implemented in the clinical setting, but limited to research studies.[87] Some studies showed that poor responsiveness to aspirin was relatively more frequent in users of enteric coated (EC) aspirin.

Plain aspirin *versus* enteric coated aspirin

EC aspirin was developed with the aim of reducing the incidence of gastrointestinal discomfort, mucosal erosions/ulcerations and bleeding, which are common complications of chronic treatment with aspirin.[83] While plain aspirin is absorbed in the stomach, EC aspirin is absorbed in the small intestine. Compared with plain aspirin, the pharmacokinetics of EC aspirin are less favorable: the time to peak maximal concentration (Tmax) is higher, while maximal concentration (Cmax) and area under the curve (AUC) are lower.[52,84] In addition, the ability of EC aspirin to inhibit platelet TxA2 production is lower,[54,85-87] especially in subjects with high body weight[88-91] or with type 2 diabetes mellitus.[92] Such inferiority in the pharmacological efficacy of EC aspirin seems to have a clinical impact, as a meta-analysis of seven randomized controlled trials of low-dose aspirin in primary cardiovascular prevention showed that the clinical efficacy of aspirin decreased with increasing body weight, particularly in subjects treated with EC aspirin.[93]

Several studies showed that EC aspirin is not safer than plain aspirin, thus disappointing the expectations that fostered its development. Endoscopic studies in asymptomatic aspirin-treated subjects showed that, compared with plain aspirin, the administration of EC aspirin is associated with fewer gastric mucosal lesions[94] but with more frequent lesions of the small bowel mucosa,[95] thus suggesting that asymptomatic gastrointestinal mucosal lesions are caused by topical effects of aspirin in the region of its absorption. Most importantly, several studies failed to provide data supporting the clinical benefit of EC aspirin in terms of prevention of gastrointestinal bleeding and ulcers,[96] which are likely caused by the systemic effects of the drug. Therefore, plain aspirin has a more favorable pharmacological profile than EC aspirin and, in my opinion, should be preferred over EC aspirin for cardiovascular prevention in all patients at risk.

Inadequate inhibition of TxA2 biosynthesis by aspirin ("aspirin resistance") in essential thrombocythemia

In the last decade, some well-conducted studies evaluated the ability of aspirin to inhibit TxA2 biosynthesis in ET patients. The published studies can be divided into two categories: the first category evaluated the pharmacological response to aspirin by measuring TxB2 production serially at 0.5-8.0 hours after aspirin ingestion, while the second category measured TxB2 production the day after the last ingestion of aspirin, thus really exploring the recovery of the ability of platelets to synthesize TxA2.

Evaluation of the pharmacological response to aspirin ("aspirin resistance")

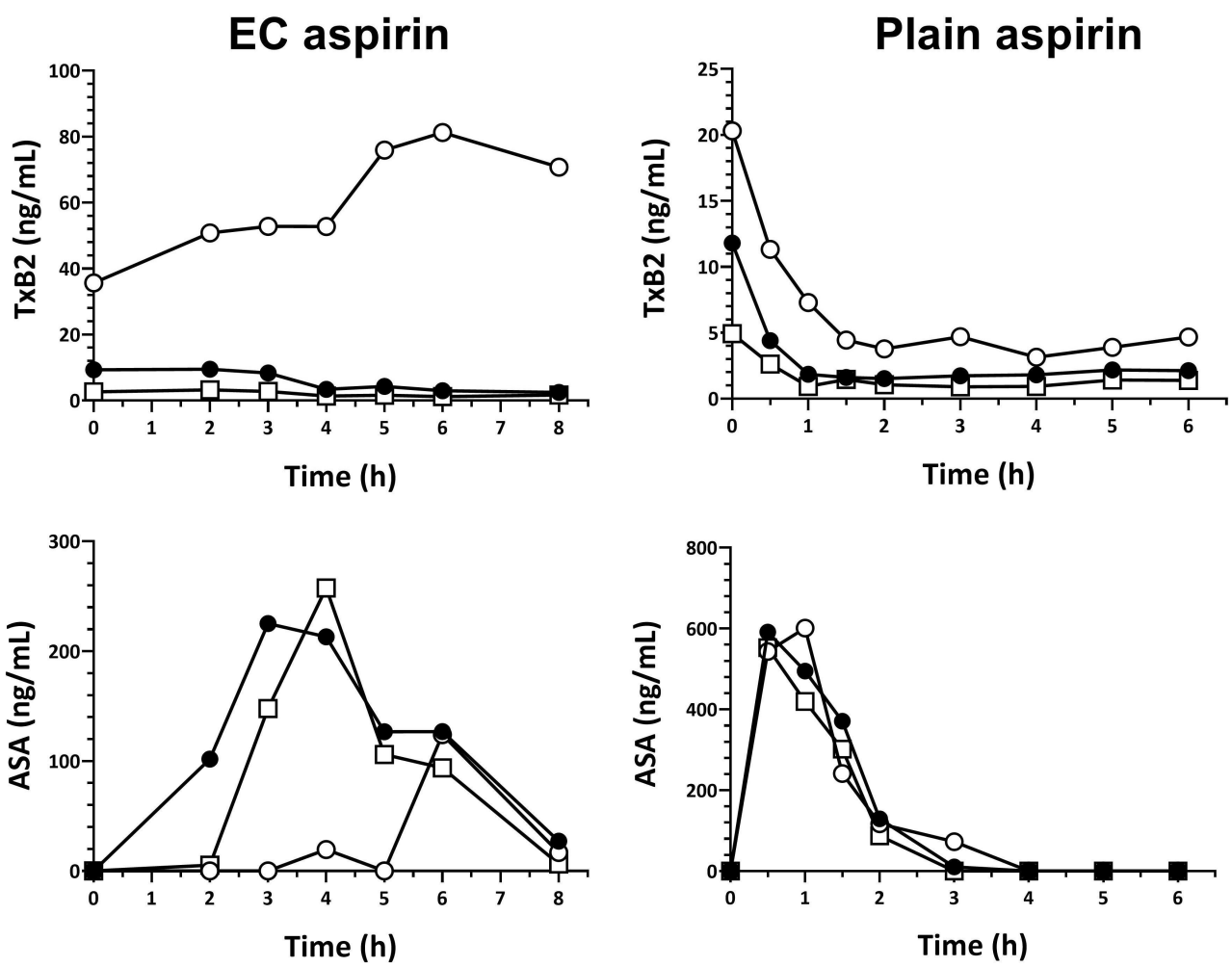
A study of aspirin pharmacokinetics and pharmacodynamics and of the potential mechanisms causing poor pharmacological responsiveness to aspirin in ET patients was published by Scavone *et al*. in 2020.[54] Seventeen ET patients on chronic treatment with 100 mg EC aspirin once daily and ten healthy subjects on 100 mg EC aspirin once daily for 7 days were enrolled. Blood samples were collected before the morning administration of aspirin (exactly 24 hours after the last dose) and between 2 and 8 hours after the morning dose. Based on their high serum TxB2 levels 6 hours after dosing (when the nadir values in healthy subjects were observed), six patients were identified as poor responders (Figure 1). All of them had plasma levels of aspirin and salicylic acid that were much lower than those of controls and ET good responders (Figure 1). Their plasma and whole blood activity of esterases were normal, thus ruling out the possibility that poor aspirin response was attributable to accelerated de-acetylation of the drug. When experiments were repeated in the same subjects using 100 mg plain aspirin instead of EC aspirin, all studied parameters were normal in all ET patients, thus suggesting that some ET patients are "resistant" to EC aspirin, but not to plain aspirin. Similar results had previously been obtained in type 2 diabetic patients[92] and in subjects with high body weight.[89-91]

Evaluation of the recovery of the ability of platelets to synthesize TxB2 after aspirin administration

In a study of 60 healthy subjects, it was shown that serum TxB2 levels measured 24 hours after the ingestion of one 325 mg aspirin tablet were highest in subjects displaying the highest percentage of reticulated platelets.[97] The ability of reticulated platelets to form TxB2 was inhibited *in vitro* by a COX-1 antagonist and, albeit less efficiently, by a COX-2 inhibitor, suggesting that newly formed platelets synthesize TxB2 through the action of uninhibited COX-1 and, partially, of COX-2,[97] which is present in immature, but not in mature platelets.[27]

A similar study in 41 ET patients and 24 healthy controls treated with EC aspirin (100 mg once daily) revealed that urinary 11-dehydro-TxB2 and total serum TxB2 levels, measured the day after the last ingestion of aspirin, were higher in ET patients than in controls.[98] Both metabolites were partially (about 25%) reduced by the *in vivo* administration of the COX-2 inhibitor etoricoxib on top of aspirin for 7 days. Similarly, *in vitro* addition of the COX-2 inhibitor NS-398 (1 μmol/L) reduced serum TxB2 levels by about 30%, which, in contrast, were completely inhibited by 50 μmol/L aspirin. Platelet COX-2 expression was increased in ET patients and correlated with circulating reticulated platelets. Therefore, this study suggested that COX-1 and, partly, COX-2 in newly formed, non-acetylated platelets are responsible for the observed high levels of TxA2 metabolites the day after aspirin ingestion by ET patients.[98]

The same group of investigators later showed in the same



	HS	ET-R	ET-PR	*p*
C max (ng/mL)	477.6 [333.2-621.5]	425.2 [372.5-672.9]	0 [0-245.4]	*0.022*
AUC (ng·h/mL)	693.8 [498.8-900.8]	820.2 [709.3-1200]	0 [0-395]	*0.004*

	HS	ET-R	ET-PR	*p*
C max (ng/mL)	593.3 [441.3-1164]	740 [559.4-1172]	952.2 [313.7-1096]	*0.826*
AUC (ng·h/mL)	850.4 [656-1047]	859.4 [638.8-1277]	924.2 [832.9-1141]	*0.613*

Figure 1. Variations in serum thromboxane B2 levels and plasma concentrations of acetylsalicylic acid following the oral administration of 100 mg enteric coated aspirin or plain aspirin to healthy subjects and patients with essential thrombocythemia. Study subjects were on treatment with once daily 100 mg enteric coated (EC) aspirin tablets (left graphs) or plain aspirin tablets (right graphs) for ≥7 days. Measurements were performed on serum or plasma samples obtained before (time 0, which corresponds to 24 hours after the previous aspirin ingestion) and at the indicated time points after the witnessed oral administration of the drug in the morning of the experiment. Open squares, healthy subjects; open circles, ET patients who were poor responders to EC aspirin (ET-PR); closed circles, ET patients who were good responders to EC aspirin (ET-R). Each point in the graphs refers to the median value obtained in ten healthy subjects, six ET-PR and ten ET-R. Interquartile ranges [IQR] are not indicated for the sake of better legibility of the graphs. The tables under the graphs indicate the median [IQR] of maximal plasma aspirin concentration (Cmax) and of the area under the curve (AUC). TxB2: thromboxane B2; ASA: acetylsalicylic acid (aspirin); HS: healthy subjects. Data were analyzed by Kruskal Wallis analysis of variance. Adapted from Scavone *et al*.[52]

41 EC aspirin-treated ET patients that there was a good correlation between the total serum TxB2 levels measured 24 hours after the last 100 mg dose of EC aspirin and the reticulated platelet count, while the correlation with reticulated platelet fraction was much weaker (Table 3).[99] In addition, in a randomized cross-over study, the authors evaluated the effects of different aspirin regimens in 21 ET patients with high total serum TxB2 levels (≥4 ng/mL) 24 hours after the administration of aspirin: EC aspirin 100 mg twice daily, EC aspirin 200 mg once daily, or plain aspirin 100 mg once daily. Compared with EC aspirin 100 mg once daily, EC aspirin 100 mg twice daily reduced 24-hour total serum TxB2 by 88%, EC aspirin 200 mg once daily by 39%, while plain aspirin 100 mg once daily did not have a statistically significant effect. The authors concluded that abnormal megakaryopoiesis accounts for the shorter-lasting inhibitory effect of aspirin in ET patients, which can be rescued by increasing the frequency of aspirin doses.[99]

The already mentioned study by Scavone *et al*. confirmed that total serum TxB2 levels 24 hours after EC aspirin (100 mg once daily) were higher in ET patients than in controls.[54] A good correlation was found between the number of reticulated platelets and the 24-hour total serum TxB2 levels. However, there was no correlation between the percentage of reticulated platelets and total serum TxB2 levels, indicating that high reticulated platelet count in ET is a reflection of increased platelet production (the hallmark of ET), rather than increased platelet turnover as has been suggested (Table 3).[10,100-103] Consistent with this interpretation was the finding that the platelet count cor-

Table 3. Coefficients of correlation between platelet count, reticulated platelet count and fraction with serum thromboxane B2 levels 24 hours after the oral administration of aspirin to patients with essential thrombocythemia.

Independent variable	Pascale *et al.*[99]		Scavone *et al.*[54]	
	Coefficient of correlation (R)	*P*	Coefficient of correlation (R)	*P*
Reticulated platelet count	0.61	<0.001	0.61	0.0018
Platelet count	na	na	0.62	0.0018
Reticulated platelet fraction	0.34*	0.03	0.03	0.88

*The mild statistically significant correlation with reticulated platelet fraction suggests that some patients had increased platelet turnover, in addition to the predominant increase in platelet production. Indeed, 12 (29.3%) of the 41 patients enrolled in the study by Pascale *et al.* had had previous thrombotic events, which are associated with increased platelet turnover in ET patients.[56,57] na: not available.

related with the 24-hour serum TxB2 levels to exactly the same extent as the reticulated platelet count (Table 3) and that no statistically significant correlation was observed between reticulated platelet count and serum TxB2 levels expressed relative to the sample platelet count (ng/10^8 platelets). The 24-hour total serum TxB2 levels were also high after treatment of the same patients with plain aspirin (albeit lower than 24 hours after EC aspirin), in contrast with the low levels (comparable to those in healthy controls) measured shortly after drug administration. This finding, which replicates the results by Pascale *et al.*,[99] is not surprising, because the initial response to aspirin cannot influence the rate of platelet production. Finally, twice daily administration of 100 mg aspirin maintained the serum TxB2 levels low also in the pre-dose (after 12 hours) samples, as already shown by Pascale *et al.*[99]

Aspirin Regimens in Essential Thrombocythemia (ARES) is an ongoing parallel-arm, placebo-controlled, randomized phase II trial in 300 ET patients. It is testing the effects of 100 mg EC aspirin twice or three times daily, compared to the usual once daily dose, on the inhibition of platelet TxA2 and vascular prostacyclin production.[102] The first phase of the trial, which was completed in 245 ET patients, showed that the twice daily regimen was superior in inhibiting TxA2 production, while the vascular production of prostacyclin was reduced by 35% in both arms. Administering aspirin three times daily did not further reduce TxA2 production and was associated with more gastrointestinal discomfort.[104]

Two mechanisms of inadequate aspirin efficacy in patients with essential thrombocythemia

To summarize, it appears that two independent mechanisms are responsible for inadequate pharmacological effects of aspirin in ET (Figure 2): poor drug absorption and increased recovery of the capacity to synthesize high levels of TxA2. Poor drug absorption was observed when EC aspirin was used and would be easily overcome by using plain aspirin, which is readily absorbed with negligible inter-individual variability.[54] Increased recovery of the capacity to synthesize high levels of TxA2 is caused by increased platelet production, rather than increased turnover, and may be overcome by administering aspirin twice daily instead of once daily.[54,99-101]

Conclusions and suggestions

Low-dose aspirin is the recommended treatment for the control of vascular events in all risk categories of ET patients (Table 2), with the exception of patients at very low risk, who should be given aspirin for treatment of vasomotor/microvascular disturbances only. A simple observational approach should be preferred over aspirin prophylaxis in low-risk patients with platelet counts >1,000x10^9/L or harboring *CALR* mutations. Cytoreduction should be preferred over aspirin prophylaxis for high-risk or symptomatic patients with platelet counts >1,000/10^9/L. Plain aspirin should be preferred over EC aspirin because some ET patients display poor responsiveness to the latter,[54] similarly to patients with type 2 diabetes mellitus and subjects weighing more than 70 Kg.[89-92] Although the clinical efficacy of the two aspirin formulations has not been compared in randomized controlled trials, the more efficient inhibition of TxA2 production by plain aspirin would predict a more efficient protection from thrombosis.[81-83] On the other hand, comparisons of the gastrointestinal toxicity of the two formulations have been performed in many studies, which indicated their equivalence, thus crippling the rationale for the use of EC aspirin in clinical practice.

When treated with the once daily aspirin regimen, most ET patients do not display virtually complete inhibition of platelet TxA2 production persisting for 24 hours, which is necessary for the prevention of thrombosis.[83,104] This phenomenon is attributable to the increased daily platelet production, which causes the presence of a high number of immature platelets with non-acetylated COX-1 in the circulation. Several studies showed that twice daily aspirin administration overcomes this problem.[54,99-103,104] The results of these studies are certainly very important and,

although additional long-term safety data may be necessary to evaluate the incidence of dose-related gastrointestinal side effects,total serum TxB2 levels could already change our clinical practice. Safety data on bleeding complications are not strictly necessary, as they would hardly be different from results of randomized controlled trials and real-world data obtained in millions of non-ET subjects treated with once daily aspirin causing virtually complete inhibition of TxA2 synthesis throughout the 24-hour dosing interval.[83,104] In addition, phase III randomized controlled trials comparing the safety and efficacy of the two aspirin dose regimens in ET would be practically impossible to organize, because of the very high number of these relatively rare patients who should be enrolled. Concerns about acquired VWF defects should influence the decision of whether to treat or not treat ET patients with aspirin:

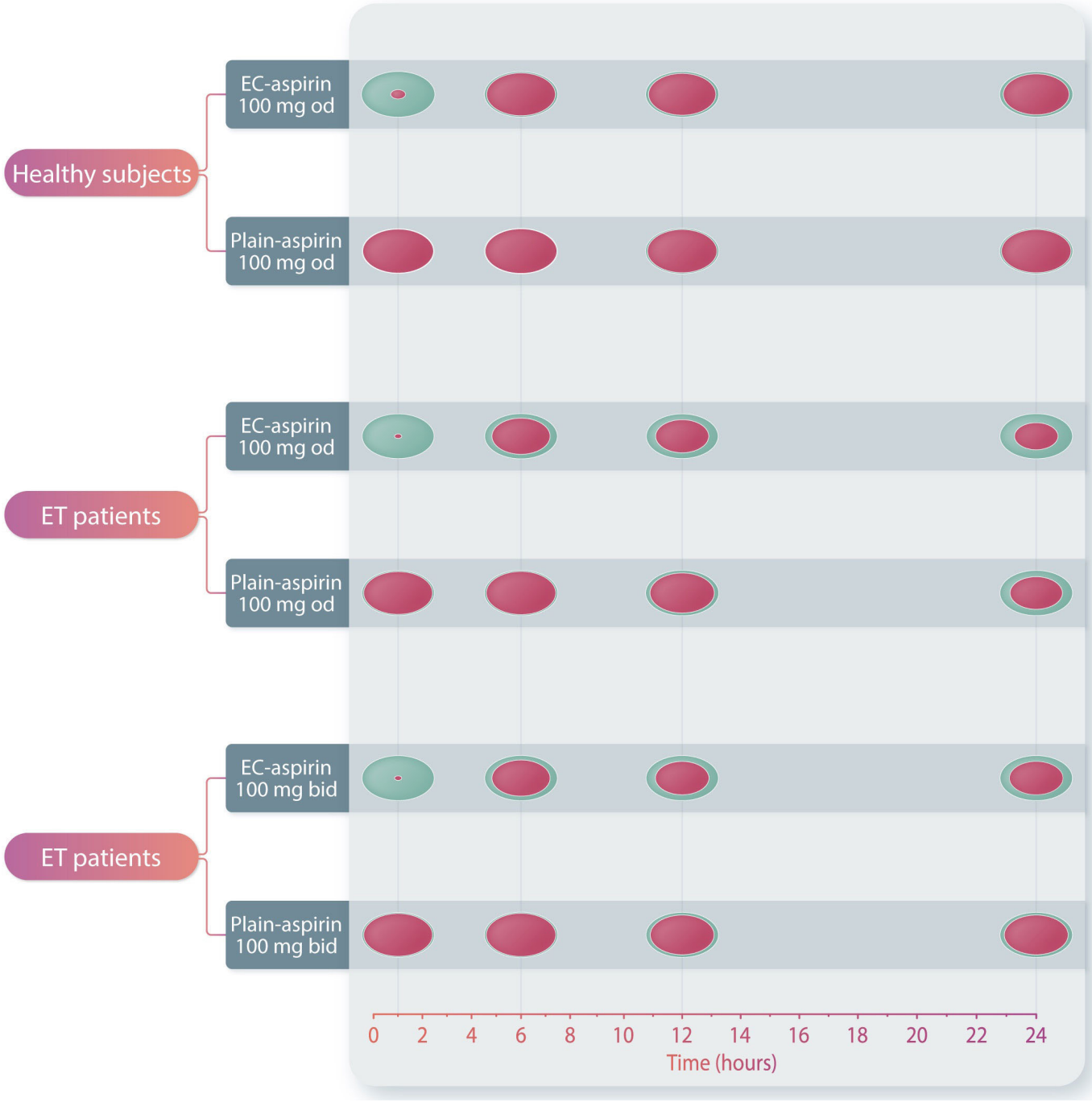


Figure 2. Schematic representation of the mean extent of inhibition of platelet thromboxane A2 production over time in healthy subjects and patients with essential thrombocythemia treated with different regimens of enteric coated aspirin or plain aspirin. Oval green symbols represent resting, non-acetylated platelets; oval red symbols inside the green ovals represent the mean percent inhibition of platelet thromboxane A2 production by aspirin. Mean percent inhibition after enteric coated (EC) aspirin is lower than that after plain aspirin in patients with essential thrombocythemia (ET) at each time point because some ET patients are not responsive to EC aspirin. The mean percent inhibition of platelets from ET patients at 24 hours after once daily (od) administration of aspirin is significantly decreased compared to that at earlier time points, because the recovery of the ability to produce thromboxane A2 by newly formed non-acetylated platelets at 24 hours is significantly increased compared to normal, due to the increased daily production of platelets in ET. The mean percent inhibition of platelets from healthy controls at 24 hours after once daily administration of aspirin is negligibly decreased compared to that at earlier time points, because some newly formed platelets are formed by megakaryocytes that have been acetylated by aspirin in the bone marrow. Twice daily (bid) administration of any formulation of aspirin to ET patients increases the percent platelet inhibition at the 24-hour time-point, compared to once daily aspirin, which is comparable to that at the 12-hour time-point because the number of newly produced platelets by megakaryocytes at 12 hours is 50% lower than after 24 hours. The indicated percentages of thromboxane A2 inhibition do not correspond exactly to actual measurements because available data from corresponding studies are not sufficient to allow reporting of accurate numbers; differences have been amplified for better clarity of this visual representation.

when the decision to use aspirin is taken, the most efficient dose regimen must be selected, because undertreatment would still carry the risk of side effects, while likely failing to be clinically effective. On the other hand, it is reasonable to predict that not all ET patients should be treated with twice daily aspirin.[54] The demonstration that recovery of the ability to produce high levels of TxA2 after aspirin ingestion in ET is a function of the patient's platelet count[54] provided evidence that, for instance, ET patients whose platelet count has been normalized by treatment do not need the twice daily regimen, which would expose them unnecessarily to a potential increased risk of gastrointestinal side effects. In contrast, the suggestion to use aspirin twice daily only in ET patients with a particularly high risk of thrombosis due to the coexistence of cardiovascular risk factors or evidence of treatment failure[10,103] is, in my opinion, questionable, because all patients with an indication for drug treatment should be given an effective drug regimen, independently of the severity of their condition.

Disclosures

No conflicts of interest to disclose.

Data-sharing statement

Data and protocols can be requested by sending an email to the author.

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