American Society of Hematology
2021 L Street NW, Suite 900,
Washington, DC 20036
Phone: 202-776-0544 | Fax 202-776-0545
editorial@hematology.org

A randomized, double-blind trial of three aspirin regimens to optimize antiplatelet therapy in essential thrombocythemia

Tracking no: BLD-2019-004596R1

Bianca Rocca (Universita Cattolica, Italy) Alberto Tosetto (S. Bortolo Hospital, Italy) Silvia Betti (Catholic University, Italy) Denise Soldati (Fondazione Policlinico Universitario A. Gemelli IRCCS, Italy) Giovanna Petrucci (Universita Cattolica, Italy) Elena Rossi (Catholic University, Fondazione Policlinico A.Gemelli, Italy) Andrea Timillero (Hematology Project Foundation, Italy) Viviana Cavalca (Centro Cardiologico Monzino, IRCCS, Milan, Italy) Benedetta Porro (Centro Cardiologico Monzino, IRCCS,, Italy) Alessandra Iurlo (Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Italy) Daniele Cattaneo (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico,) Cristina Bucelli (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Italy) Alfredo Dragani (Rare Blood Disease Center, Italy) Mauro Di Ianni (University of Chieti-Pescara, Italy) Paola Ranalli (Santo Spirito Hospital, Italy) Francesca Palandri (Institute of Hematology, Italy) Nicola Vianelli ((Institute of Hematology, Italy) Eloise Beggiato (A.O.U. Città della Salute e della Scienza di Torino, Italy) Giuseppe Lanzarone (University of Turin, Italy) Marco Ruggeri (Hematology, San Bortolo Hospital, Vicenza, Italy, Italy) Giuseppe Carli (San Bortolo Hospital, Italy) Elena Elli (San Gerardo Hospital, Italy) Monica Carpenedo (ASST Ospedale San Gerardo di Monza, Italy) Irene Bertozzi (University of Padova, Italy) Chiara Paoli (University of Florence, Italy) Maria Randi (University of Padua, Italy) Alessandra Ricco (Hematology Section, University of Bari, Italy) Giorgina Specchia (University of Bari, Italy) Alessandro Vannucchi (University of Florence, Italy) Francesco Rodeghiero (Hematology Project Foundation, Italy) Carlo Patrono (Catholic University of Rome, Italy) Valerio De Stefano (Catholic University, Fondazione Policlinico A. Gemelli IRCCS, Italy)

Abstract:
Essential thrombocythemia (ET) is characterized by abnormal megakaryopoiesis and enhanced thrombotic risk. Once-daily (od), low-dose aspirin is the recommended antithrombotic regimen, but accelerated platelet generation may reduce the duration of platelet cyclooxygenase (COX)-1 inhibition. We performed a multicenter, double-blind trial to investigate the efficacy of three aspirin regimens in optimizing platelet COX-1 inhibition while preserving COX-2-dependent vascular thromboresistance. Two-hundred-forty-five patients on chronic od low-dose aspirin were randomized (1:1:1) to receive 100 mg aspirin od, twice-daily, bid), or three-times daily (tid) for 2 weeks. Serum thromboxane B_2 ($sTXB_2$), a validated biomarker of platelet COX-1 activity, and urinary prostacyclin metabolite (PGIM) excretion were measured at randomization and after 2 weeks, as primary surrogate endpoints of efficacy and safety, respectively. Urinary TX metabolite (TXM) excretion, gastrointestinal tolerance, and ET-related symptoms were also investigated. Evaluable patients assigned to the bid and tid regimens showed substantially reduced inter-individual variability and lower median values of $sTXB_2$: 19.3[9.7-40], 4 [2.1-6.7], and 2.5[1.4-5.65] ng/ml in the od (n=85), bid (n=79) and tid (n=79) arms, respectively. Urinary PGIM was comparable in the three arms. Urinary TXM was significantly reduced by 35% in both experimental arms. Patients in the tid arm reported a higher abdominal discomfort score. In conclusion, the currently recommended aspirin regimen of 75-100 od for cardiovascular prophylaxis appears largely inadequate in reducing platelet activation in the vast majority of ET patients. The antiplatelet response to low-dose aspirin can be markedly improved by shortening the dosing interval to 12 hours, with no improvement by further reducing it. (EudraCT 2016-002885-30)

Conflict of interest: COI declared - see note

COI notes: Valerio De Stefano has received consulting and lecture fees from Amgen, Bayer, Celgene, Novartis, and institutional research grants from Novartis; Elena Maria Elli received advisory board and project fees from Novartis; Francesca Palandri has received consulting and lecture fees from Novartis; Carlo Patrono has received consulting and lecture fees from Acticor Biotech, Amgen, Bayer, GlaxoSmithKline and Zambon, and institutional research grants from Bayer; he serves as Chairperson of the Scientific Advisory Board of the International Aspirin Foundation; Bianca Rocca has received consulting and lecture fees from Bayer AG and Novartis; Francesco Rodeghiero received consulting and lecture fees and institutional research grants from Novartis and Amgen; Alessandro Maria Vannucchi has received consulting and lecture fees from Italfarmaco, Novartis, Shire, Celgene. All the other Authors declared no competing interests.

Preprint server: No;

Author contributions and disclosures: Author contribution and disclosures VDS, BR, AT, FR, CP designed the study, analysed data and drafted the paper DS, AT coordinated the study GP, VC, BP measured serum and urine biomarkers All the other Authors performed the research by recruiting patients, collecting data and samples. Valerio De Stefano has received consulting and lecture fees from Amgen, Bayer, Celgene, Novartis, and institutional research grants from Novartis; Elena Maria Elli received advisory board and project fees from Novartis; Francesca Palandri has received consulting and lecture fees from Novartis; Carlo Patrono has received consulting and lecture fees from Acticor Biotech, Amgen, Bayer, GlaxoSmithKline and Zambon, and institutional research grants from Bayer; he serves as Chairperson of the Scientific Advisory Board of the International Aspirin Foundation; Bianca Rocca has received consulting and lecture fees from Bayer AG and Novartis; Francesco Rodeghiero received consulting and lecture fees and institutional research grants from Novartis and Amgen; Alessandro Maria Vannucchi has received consulting and lecture fees from Italfarmaco, Novartis, Shire, Celgene. All the other Authors declared no competing interests.

Non-author contributions and disclosures: No;

Agreement to Share Publication-Related Data and Data Sharing 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 kept available at www.osf.io (Foster ED, Deardorff A (2017) Open Science Framework (OSF). Journal of the Medical Library Association : JMLA 105 (2):203-206) upon request. Requests for access should be sent to alberto.tosetto@aulss8.veneto.it, and will be subjected to the revision and approval of the Steering Committee of the study. The study protocol is included as a data supplement available with the online version of this article.

Clinical trial registration information (if any): EudraCT 2016-002885-3

A randomized, double-blind trial of three aspirin regimens to optimize antiplatelet therapy in essential thrombocythemia

Bianca Rocca[1§], Alberto Tosetto[2§], Silvia Betti[3], Denise Soldati[3], Giovanna Petrucci[1], Elena Rossi[3,4], Andrea Timillero[5], Viviana Cavalca[6], Benedetta Porro[6], Alessandra Iurlo[7], Daniele Cattaneo[7], Cristina Bucelli[7], Alfredo Dragani[8], Mauro Di Ianni[8], Paola Ranalli[8], Francesca Palandri[9], Nicola Vianelli[9], Eloise Beggiato[10], Giuseppe Lanzarone[10], Marco Ruggeri[2], Giuseppe Carli[2], Elena Maria Elli[11], Monica Carpenedo[11], Irene Bertozzi[12], Chiara Paoli[13], Maria Luigia Randi[12], Alessandra Ricco[14], Giorgina Specchia[14], Alessandro Maria Vannucchi[13], Francesco Rodeghiero[5], Carlo Patrono[1] and Valerio De Stefano[3,4]

on behalf of the Aspirin Regimens in EsSential thrombocythemia (ARES) Investigators.

[1]Section of Pharmacology, Catholic University School of Medicine, Rome; [2]Hematology Department, Ospedale San Bortolo, Vicenza; [3]Fondazione Policlinico Universitario A. Gemelli, IRCCS, Roma;[4]Department of Radiological and Hematological Sciences, Section of Hematology, Catholic University School of Medicine, Roma; [5]Hematology Project Foundation, Vicenza; [6]Centro Cardiologico Monzino, IRCCS, Milano; [7]Hematology Division, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, and University of Milano, Milano; [8]Hematology Department, S. Spirito Hospital, Pescara; [9]Institute of Hematology "L. and A. Seràgnoli", S. Orsola-Malpighi Hospital, Bologna; [10]Unit of Hematology, Department of Oncology, University of Torino, Torino; [11] Division of Haematology and Bone Marrow Transplantation Unit, Ospedale San Gerardo, ASST Monza; [12]Department of Medicine - DIMED, University of Padova, Padova; [13]CRIMM-Center of Research and Innovation of Myeloproliferative Neoplasms, Azienda Ospedaliera Universitaria Careggi, and Dept Experimental and Clinical medicine, University of Firenze, Firenze; [14]Department of Emergency and Organ Transplantation (D.E.T.O.), Hematology Section, University of Bari, Bari; all in Italy.

[§]Bianca Rocca and Alberto Tosetto contributed equally to this work.

Short Title: Aspirin Regimens in Essential Thrombocythemia

Category:

Word count: 3130

Key words: aspirin, essential thrombocythemia, thromboxane, cyclooxygenase, prostacyclin

Corresponding Author:

Valerio De Stefano, MD
Department of Radiological and Hematological Sciences,
Section of Hematology,
Catholic University,
Fondazione Policlinico Universitario A. Gemelli IRCCS,
Largo Gemelli, 8
00168 Rome, Italy
Phone: +39-06-30154180
e-mail: valerio.destefano@unicatt.it

Key points

• The majority of 245 patients with essential thrombocythemia treated with once-daily, low-dose aspirin display incomplete platelet inhibition

• In this randomized study platelet inhibition was improved by a twice-daily dosing interval with no further inhibition by a shorter interval

Abstract

Essential thrombocythemia (ET) is characterized by abnormal megakaryopoiesis and enhanced thrombotic risk. Once-daily (od), low-dose aspirin is the recommended antithrombotic regimen, but accelerated platelet generation may reduce the duration of platelet cyclooxygenase (COX)-1 inhibition.

We performed a multicenter, double-blind trial to investigate the efficacy of three aspirin regimens in optimizing platelet COX-1 inhibition while preserving COX-2-dependent vascular thromboresistance. Two-hundred-forty-five patients on chronic od low-dose aspirin were randomized (1:1:1) to receive 100 mg aspirin od, twice-daily, bid), or three-times daily (tid) for 2 weeks. Serum thromboxane B_2 ($sTXB_2$), a validated biomarker of platelet COX-1 activity, and urinary prostacyclin metabolite (PGIM) excretion were measured at randomization and after 2 weeks, as primary surrogate endpoints of efficacy and safety, respectively. Urinary TX metabolite (TXM) excretion, gastrointestinal tolerance, and ET-related symptoms were also investigated.

Evaluable patients assigned to the bid and tid regimens showed substantially reduced inter-individual variability and lower median values of $sTXB_2$: 19.3[9.7-40], 4 [2.1-6.7], and 2.5[1.4-5.65] ng/ml in the od (n=85), bid (n=79) and tid (n=79) arms, respectively. Urinary PGIM was comparable in the three arms. Urinary TXM was significantly reduced by 35% in both experimental arms. Patients in the tid arm reported a higher abdominal discomfort score.

In conclusion, the currently recommended aspirin regimen of 75-100 od for cardiovascular prophylaxis appears largely inadequate in reducing platelet activation in the vast majority of ET patients. The antiplatelet response to low-dose aspirin can be markedly improved by shortening the dosing interval to 12 hours, with no improvement by further reducing it. (EudraCT 2016-002885-30)

Introduction

Essential Thrombocythemia (ET) is a chronic Philadelphia-negative myeloproliferative neoplasm (MPN) characterized by clonal thrombocytosis and enhanced risk of arterial and venous thrombosis,[1-5] with ≈20% of patients presenting with mainly arterial thrombosis at diagnosis.[3] The incidence of thrombosis during the course of the disease averages 2% per year.[1,6] Thrombosis recurrence reaches up to 8% per year.[2] Increased thromboxane (TX)A_2 biosynthesis has been reported in ET patients,[1,7-9] suggesting a potential link between persistently enhanced platelet activation and vascular complications.[2]

Current recommendations for low-dose aspirin in ET patients are largely based on aspirin trials in non-MPN subjects, as well as on a phase-3 trial in polycythemia vera (PV).[10] However, the efficacy of aspirin for cardiovascular prevention in ET has been questioned,[6] and appears limited to subgroups of patients based on retrospective and observational studies with intrinsic limitations and low quality of evidence.[11-13]

Using the same aspirin dose (75-100 mg) and dosing regimen (once daily [od]) for ET patients as for non-ET patients, implies assuming similar aspirin pharmacodynamics in both populations. Aspirin permanently acetylates platelet and megakaryocyte cyclooxygenase (COX)-1, the enzyme catalyzing the first committed step in TXA_2 biosynthesis.[14] Under physiological megakaryopoiesis, the irreversible nature of COX-1 inactivation in the bone marrow progenitors leads to a new platelet progeny with largely non-functioning enzyme throughout the 24-hour dosing interval.[14,15] However, accelerated formation and release of platelets with unacetylated COX-1 and/or COX-2 in ET[8] has been suggested to impair inhibition and fasten recovery of TXA_2-dependent platelet function during the 24-hour aspirin dosing interval.[1,15] Consistent with this hypothesis, two independent studies of relatively small sample size have reported incomplete inhibition of platelet TXA_2 biosynthesis by a standard aspirin regimen in ≥80% of ET patients.[16,17]

The antiplatelet effect of low-dose aspirin could be improved by more frequent dosing, i.e. every 12 hours (bis-in-die [bid]) more effectively than by doubling the once-daily dose.[16,17] Therefore, multiple daily dosing has been suggested to represent a more effective antiplatelet strategy in ET.[15-17] However, this hypothesis has never been tested in a large study. Moreover, the potential inhibitory effect of more frequent aspirin dosing on vascular prostacyclin (PGI_2) biosynthesis should also be considered,[18] given the enhanced thrombotic risk of ET patients.[1] Under physiological shear stress conditions, the COX-2 isozyme of vascular endothelial cells largely drives PGI_2 biosynthesis, which has clinically relevant vasorelaxant and platelet-inhibiting effects, as suggested by the cardiovascular toxicity of COX-2 inhibitors.[19] Low-dose aspirin has been shown to have limited inhibitory effects on *in vivo* PGI_2 biosynthesis, possibly because of differential rates of recovery of endothelial COX-2 vs. platelet COX-1 during the 24-hour dosing interval.[20-22] However, it is unknown whether multiple aspirin dosing may reduce endothelial PGI_2 production in patients with ET.

To address the unmet need of an optimized antiplatelet regimen in ET patients, we conceived the Aspirin Regimens in Essential Thrombocythemia (ARES) phase-2 trial.[23] Here we report the results of the dose-finding component of the ARES trial which addressed in a randomized, double-blind fashion the following main objectives: i) to define the most effective dosing regimen of aspirin administration to suppress platelet COX-1 activity in patients with ET, as reflected by a surrogate biomarker of aspirin efficacy, i.e., *ex vivo* TXB_2 production during whole blood clotting;[24,25] ii) to assess the vascular safety of an improved aspirin regimen as reflected by a biomarker of endothelial COX-2 activity, i.e., urinary PGI_2 metabolite (PGIM) excretion.[26]

Patients and methods

Design of the study

The rationale, design, main objectives, inclusion/exclusion criteria of the ARES trial (EudraCT 2016-002885-30) have been detailed elsewhere.[23] The present report relates to the dose-

finding component of ARES, consisting of a multi-center, randomized, parallel-arm, double-blind, controlled study. Eligible ET patients[23] on chronic aspirin (100 mg od) for primary or secondary cardiovascular prevention were enrolled. At Visit 1, all patients were instructed to take their usual aspirin tablet at breakfast (7-9 am) for 7-10 consecutive days. Upon run-in completion, patients were randomized 1:1:1 to enteric-coated aspirin (Cardioaspirin®, Bayer Italy) 100 mg od (breakfast), bid (every 12±2 hours, i.e. breakfast and dinner), or three times daily (tid, every 6±2 hours, i.e. breakfast, luch and dinner) for two weeks (**Figure 1**) and matching placebo, so that all patients took a tid regimen, including aspirin with or without placebo tablets according to the randomized arm. Therefore, aspirin or matching placebo tablets were taken at breakfast (7-9 am), lunch (1-2 pm), and after dinner (8-9 pm). The prescribed cytoreductive regimen, if any, was kept unchanged for the 2-week study period. At randomization (Visit 2) and after two weeks of randomized treatment (Visit 3), patients underwent blood and urine sampling in a fasting state before the next aspirin dosing (**Figure 1**). Then, patients resumed their open-label aspirin regimen (100 mg od). The Ethics Committee of the Fondazione Policlinico Universitario A. Gemelli IRCCS approved the study (protocol # 28371/16, ID 1285, final approval August 2, 2016). All participating Institutions approved the protocol. All patients signed an informed consent. The study was conducted between December 2017 and July 2018.

The co-primary endpoints were: the pharmacodynamic efficacy of the aspirin regimens, as reflected by residual serum TXB_2, and the vascular safety, as assessed by urinary PGIM excretion.

The secondary endpoint was the urinary excretion of a stable enzymatic metabolite of TXA_2/TXB_2, 11-dehydro-TXB_2 (TXM), reflecting the actual rate of *in vivo* TXA_2 biosynthesis.[27] Gastrointestinal (GI) tolerance and microvascular symptoms were also recorded during the week preceding Visit 3 with ad-hoc questionnaires: the severity of dyspepsia assessment (SODA) questionnaire, previously validated for patients taking nonsteroidal anti-inflammatory drugs (NSAIDs),[28,29] a validated questionnaire assessing MPN-related symptoms,[30] and a patient's self-

scored pain numeric rating scale (PNRS) for erythromelalgia of hands and feet ranking from 0 (no pain) to 10 (worst imaginable pain).

Methods

Routine hematochemical analyses and the mutational profile of the patients were performed in the laboratories of each participating Institution. Clinical and laboratory characteristics of the patients were collected through Research Electronic Data Capture (REDCap).[31]

Patients were randomized using a randomization list stratified by sex and participating Center, implemented within the REDCap software.

The thrombotic risk was assessed according to the International Prognostic Score of Thrombosis in Essential Thrombocythemia (IPSET-thrombosis) system, a validated prognostic score that includes age, previous thrombosis, cardiovascular risk factors, and the JAK2 V617F mutation.[32] Compliance was assessed at Visit 3 by pill counting and reviewing the patient's daily diary, where patients recorded daily timing of tablet intake, any drug other than their usual therapy and any symptom or comment that they deemed relevant.

For serum TXB_2 measurements, peripheral venous blood was collected without anticoagulant, incubated within 5 minutes[33] for 1 hour at 37° C, centrifuged 10 minutes at 1,200g, and the supernatant serum was stored at -40°C until assayed.[23] Serum TXB_2 was measured by a previously described, liquid chromatography-tandem mass spectrometry (LC-MS/MS)-validated immunoassay.[24,33,34]

The major urinary PGIM, 2,3-dinor-6-keto-$PGF_{1\alpha}$,[35] was measured by LC-MS/MS method, as previously described.[22] The major urinary TXM, 11-dehydro-TXB_2, was measured in 1-ml urine samples by a GC/MS-validated immunoassay.[36,37] Urinary prostanoid values were expressed as pg/mg of urinary creatinine, measured by a commercial kit (Creatinine Colorimetric Detection Kit; Enzo Life Sciences, Farmingdale, NY).

Statistical analyses

Based on previous findings[8,17] we assumed that the mean ± standard deviation (SD) of serum TXB_2 in ET patients on aspirin 100 mg od would approximate 22±33 ng/ml. We planned to test two hypotheses: i) the 100 mg bid regimen is more effective than 100 mg od, yielding a ≥50% reduction in serum TXB_2, and ii) the 100 mg tid regimen is more effective than 100 mg bid, yielding a ≥50% reduction in serum TXB_2. Testing these hypotheses with an α-error of 0.05 and a β-error of 0.2 (80% power) required 70 patients per treatment arm. Anticipating a 30% dropout, we estimated that 100 patients in each arm would ensure adequate statistical power. For the co-primary endpoint of urinary PGIM, the study had 80% power to test the hypothesis that any experimental aspirin regimen would reduce urinary PGIM by >30% as compared with the standard regimen. This threshold of urinary PGIM reduction was selected based on the following considerations: urinary PGIM is minimally affected (20-40% variation) by aspirin 75-100 mg daily in healthy subjects; this threshold corresponds to the intra-subject coefficient of variation upon repeated measurements of PGIM excretion over time; traditional NSAIDs, including high-dose aspirin, reduce urinary PGIM excretion by 60-80%.[26]

Differences between qualitative and quantitative variables were tested with the chi-square and Wilcoxon signed-rank tests, respectively. A linear regression model was used to evaluate possible differences in serum TXB_2 response in effect of platelet count and cytoreductive therapy. The R statistical software version 3.6.1 was used for data analysis and plotting.[38]

Data Sharing 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 kept available at www.osf.io,[39] upon request. Requests for access should be sent to alberto.tosetto@aulss8.veneto.it, and will be subjected to review and approval by the Steering Committee of the study. The study protocol is included as a data supplement available with the online version of this article.

Results

Two-hundred and fifty-one eligible, aspirin-treated (the vast majority for primary prevention, see Table 1), consenting ET patients were enrolled and started the run-in phase. Six patients withdrew their consent during this phase for personal reasons, thus 245 patients underwent randomization at Visit 2 (**Figure 2**). The demographic, clinical, and laboratory characteristics of these patients are detailed in **Table 1.** There were no statistically significant differences among the three treatment groups. One patient assigned to aspirin 100 mg od exited the study before Visit 3 for abdominal pain, and one patient had no serum sample available at Visit 3 (**Figure 2**). Thus, 243 patients were evaluable at the end of the study and were included in the analyses.

Compliance at Visit 3, as assessed by pill count and patient's diary, is reported in **Table 2**: 218 out of 243 patients (90%) took all nine pills in the three days preceding Visit 3 and were considered fully compliant. None of the patients reported taking any NSAID in the three days preceding Visit 3.

Co-primary endpoints

Serum TXB_2 level at Visit 2 averaged 19 [3.4-140.4] ng/ml (median and interquartile range; n=245) and was similar across the three treatment arms (**Table 3**). As shown in **Figure 3**, serum TXB_2 at Visit 2 displayed a substantial interindividual variability, spanning two to three orders of magnitude, with the vast majority of ET patients showing evidence of incomplete platelet COX-1 inactivation.

After two weeks of randomized aspirin treatment, serum TXB_2 values of patients assigned to either the 100 mg bid or tid regimen were reduced by 80 to 90% versus their baseline values and were significantly lower than serum TXB_2 values of patients assigned to 100 mg od (**Figure 3** and **Table 3**). In the latter group, serum TXB_2 values showed remarkably similar inter-individual variability before and after 2-week treatment (**Figure 3A**), indicating the stability of the poor

aspirin responsiveness phenotype in ET. Patients assigned to the bid (**Figure 3B**) and tid (**Figure 3C**) regimens showed substantially and significantly reduced inter-individual variability in addition to lower median values of serum TXB_2 (**Table 3**). Data were also analyzed as the individual ratio of serum TXB_2 values at Visit 3 versus Visit 2, considering that all patients at Visit 2 were on aspirin 100 mg od (**Figure 3**). This analysis was performed to minimize the effect of variables such as the platelet count, turnover rate, and body weight that are known to influence aspirin responsiveness.[17,40] In fact, we found that there was a slight but statistically significant effect of the platelet count on the response to bid and tid dosing (β coefficient: -0.02, for every 100 $x10^9$/L platelet increase, p=0.049). We found no effect of cytoreduction (β coefficient: -0.06, p=0.23). Patients randomized to the od regimen had a serum TXB_2 Visit 3:Visit 2 ratio averaging 1.03 (**Table 3**), indicating no appreciable short-term change in platelet COX-1 inhibition. The Visit 3:Visit 2 ratio of the bid and tid regimens averaged 0.14 and 0.13, respectively **(Figure 4** and **Table 3),** consistent with comparable, profound suppression of residual platelet TXA_2 production by both experimental aspirin regimens.The improved pharmacodynamic response was independent of previous thrombosis (data not shown).

Urinary PGIM excretion, a non-invasive index of endothelial COX-2 activity,[18] was similar across the treatment groups at Visit 2 (**Table 3**) and was not affected by either experimental regimen as compared to the respective baseline excretion rate, to any statistically significant extent (**Table 3** and **Figure 5**).

Secondary endpoints

Baseline urinary TXM excretion, a non-invasive index of platelet activation,[18] averaged 428 [158.8-1063.7] pg/mg creatinine (n=245), without significant differences among the three treatment groups (**Table 3**). As shown in **Figure 6**, urinary TXM at Visit 2 displayed substantial interindividual variability, spanning one to two orders of magnitude, as would be expected from patients with variably and incompletely reduced TXA_2 biosynthesis.[34] After two weeks of

randomized aspirin treatment, urinary TXM excretion rates of patients assigned to either the 100 mg bid or tid regimen were similarly reduced by 30 to 40% versus their baseline values (**Figure 6 B** and **C**), with reduced interindividual variability, and were significantly lower than TXM excretion of patients assigned to 100 mg od (**Table 3**). In the od group, TXM values were remarkably superimposable between Visit 2 and 3 (**Figure 6 A**), confirming the stability of the rate of platelet activation *in vivo*. Moreover, there was a positive, significant association between individual serum TXB_2 ratios at Visit3/Visit 2 and the corresponding urinary TXM Visit 3/Visit 2 ratios (**Figure 7**) (correlation coefficient, $r^2=0.12$ $p<0.0001$). Therefore, optimization of aspirin pharmacodynamics results in reduced *in vivo* platelet activation in ET patients.

Two-hundred and thirty-nine patients (98%) completed the SODA[28], the MPN Symptom Assessment Form (MPN-SAF) questionnaire [30], and the PNRS for erythromelalgia at Visits 2 and 3. Patients in the aspirin 100 mg tid group showed a significantly higher score of GI disturbances as compared to the other groups (**Supplemental Table 1**), even though none of the patients experienced GI adverse events requiring medical intervention. No major differences were observed in the microvascular disturbance scores (**Supplemental Tables 2** and **3**), except for one query related to sleeping difficulties that were apparently reduced in the bid arm (**Supplemental Tables 2** and **3**).

There were no major bleeding (defined according to the International Society of Haemostasis and Thrombosis)[41] nor adverse cardiovascular events during the 2-week randomized treatment, as well as during the following 2 weeks of observation after Visit 3.

Discussion

The way in which low-dose aspirin prevents atherothrombosis is through permanent inactivation of platelet COX-1, resulting in virtually complete (i.e. >97%) suppression of TXA_2

production throughout the 24-hour dosing interval.[20] There is consistency in the saturability of the acetylation of platelet COX-1,[42] suppression of TXA_2 formation[43] and reduction in atherothrombotic events at daily doses of aspirin in the range of 75 to 100 mg.[20] Although the clinical efficacy of low-dose aspirin has been evaluated in subjects at variable risk of vascular occlusion, spanning the whole spectrum from asymptomatic, healthy subjects[44] to patients with acute ischemic syndromes,[45] its use in MPNs has been largely based on extrapolation from non-MPN trials and from a single trial in PV.[10] In the absence of any aspirin trial in ET patients, justification for its use based on extrapolation from other clinical settings would require demonstrating comparable pharmacodynamic response (i.e., platelet TXA_2 suppression) in ET and non-ET subjects. We reported preliminary evidence for aspirin-resistant platelet TXA_2 production in ET patients,[8] and showed that aspirin responsiveness could be rescued -at least in part- by shortening the dosing interval.[17] We suggested that this reversible phenotype of biochemical "resistance" could be explained by accelerated renewal of the drug target (i.e., platelet COX-1) because of pathological megakaryopoiesis.[17]

We designed the ARES study with two main objectives: i) to demonstrate improved antiplatelet efficacy and preserved endothelial safety of an optimized aspirin dosing regimen for both primary and secondary prevention in a large population of ET patients; ii) to assess long-term compliance with and tolerability of the selected regimen.[23] The present report deals with the results of the first component of the study.

We found high absolute values and marked interindividual variability in serum TXB_2, a validated biomarker of low-dose aspirin efficacy.[24,25] with the vast majority of ET patients displaying biochemical evidence of inadequate platelet inhibition when treated with a standard low-dose aspirin regimen. In fact, only ≈5% of non-ET subjects in previous studies had serum TXB_2 levels >10 ng/ml at 24 hours after dosing, corresponding to <97% COX-1 inhibition,[34,46] as compared to 72% of ET patients of the present study on the same once-daily aspirin regimen. It should be emphasized that most traditional NSAIDs (with the possible exception of high-dose

naproxen),[47] inhibit platelet TXA_2 production by <95%, which would correspond to a residual serum TXB_2 >15-30 ng/ml (depending on platelet count), a level comparable to the average basal value (19 ng/ml) measured in our aspirin-treated ET patients. Incomplete platelet COX-1 inhibition by NSAIDs has been shown to be insufficient to exert a cardioprotective effect, and to protect against COX-2-dependent cardiotoxicity.[48]

We could demonstrate with high statistical confidence that a bid regimen of aspirin administration reduced interindividual variability in serum TXB_2 and lowered by ≈90% the residual serum TXB_2 level. However, no further improvement in antiplatelet pharmacodynamics was achieved by a tid regimen, suggesting that a ceiling effect was reached in matching accelerated renewal of the drug target with a shortened dosing interval. Both experimental regimens similarly reduced *in vivo* TXA_2-dependent platelet activation, as reflected by urinary TXM excretion, consistent with saturability of platelet COX-1 inactivation with a bid regimen of aspirin administration in ET. The apparent endothelial safety of such a regimen in sparing PGI_2 biosynthesis confirms the preliminary findings in a small sample of ET patients,[22] apparently at odds with earlier findings in healthy subjects.[26] Whether the markedly different study design and size and/or the enteric-coated vs plain aspirin formulation used in these studies account for the apparent discrepancy remains to be established.

Based on the present results, we have chosen aspirin 100 mg bid as the experimental regimen to be compared with the standard 100 mg od regimen for maintenance of superior antiplatelet efficacy, compliance and tolerability in the long-term phase of the ARES study, in which the same ET patients are being re-randomized to one of the two aspirin regimens.

We conclude that: i) the currently recommended aspirin regimen of 75-100 od for primary or secondary cardiovascular prophylaxis is largely inadequate in reducing platelet activation in the vast majority of ET patients; ii) the antiplatelet response to low-dose aspirin can be markedly improved by shortening the dosing interval to 12 hours, with no significant improvement by further

reducing it; iii) the long-term superiority, compliance and tolerability of an optimized aspirin regimen remains to be investigated in the ongoing phase of the ARES trial.[22]

Acknowledgements

The ARES trial has been funded by the Italian Medicines Agency (AIFA), study code FARM12Y8H. Aspirin and placebo employed in the trial were generously provided by Bayer AG, through the courtesy of Drs. Elmar Detering and Paolo Montanari.

Author contribution and disclosures

VDS, BR, AT, FR, CP designed the study, analysed the data and drafted the paper

DS, AT coordinated the study

GP, VC, BP measured serum and urine biomarkers

All the other Authors contributed to the research by recruiting patients, collecting data and samples.

Valerio De Stefano has received consulting and lecture fees from Amgen, Bayer, Celgene, Novartis, and institutional research grants from Novartis; Elena Maria Elli received advisory board and project fees from Novartis; Francesca Palandri has received consulting and lecture fees from Novartis; Carlo Patrono has received consulting and lecture fees from Acticor Biotech, Amgen, Bayer, GlaxoSmithKline and Zambon, and institutional research grants from Bayer; he serves as Chairperson of the Scientific Advisory Board of the International Aspirin Foundation; Bianca Rocca has received consulting and lecture fees from Bayer AG and Novartis; Francesco Rodeghiero received consulting and lecture fees and institutional research grants from Novartis and Amgen; Alessandro Maria Vannucchi has received consulting and lecture fees from Italfarmaco, Novartis, Shire, Celgene. All the other Authors declared no competing interests.

Figure Legends

Figure 1. Design of the study. The diagram details the design, visits and biological sample collection of the first phase of the ARES trial.

Figure 2. The CONSORT diagram of the study. The diagram depicts the trial profile.

Figure 3. Individual serum thromboxane (TX)B_2 values according to the randomized treatment. Each panel depicts on the left side the individual values of serum TXB_2 at randomization (Visit 2) and at the end of two-week treatment (Visit 3); the right side of each panel shows the corresponding distribution of the data. Panel A: once daily regimen; Panel B: twice daily regimen; Panel C: three times daily regimen.

Figure 4. Individual ratios of serum thromboxane (TX)B_2 values. Individual ratios of serum TXB_2 values measured at Visit 3 versus Visit 2 are represented for each treatment arm on the left side; the corresponding data distribution is represented on the right side of the figure.

Figure 5. Individual urinary PGI_2 metabolite (PGIM) values according to the randomized treatment. Each panel depicts on the left side the individual values of urinary PGIM excretion at Visits 2 (randomization) and 3 (end of treatment); the right side of each panel shows the corresponding distribution of the data. Panel A: once daily regimen; Panel B: twice daily regimen; Panel C: three times daily regimen.

Figure 6. **Individual urinary thromboxane metabolite (TXM) values according to the randomized treatment.** Each panel depicts on the left side the individual values of urinary TXM excretion at Visits 2 (randomization) and 3 (end of treatment); the right side of each panel shows the corresponding distribution of the data. Panel A: once daily regimen; Panel B: twice daily regimen; Panel C: three times daily regimen.

Figure 7. Correlation between Visit 3/Visit 2 individual ratios of serum TXB_2 and urinary TXM values. The plot shows the linear correlation between the ratios of serum TXB_2 and urinary TXM values at Visit 3 / Visit 2 in all patients, according to the randomized treatment; correlation coefficient, $r^2=0.12$ $p<0.0001$.

Table 1. Characteristics of 245 randomized ET patients overall and according to the assigned treatment.

	All N=245	**100 mg od N=86**	**100 mg bid N=79**	**100 mg tid N=80**
Sex:				
Male, n (%)	112 (45.7)	40 (46.5)	36 (45.6)	36 (45)
Female, n (%)	133 (54.3)	46 (53.5)	43 (54.4)	44 (55)
Age at diagnosis (years)	53 [42-63]	52 [41.2-62.8]	59 [43.5-65.5]	48.5 [39.8-58]
Age at enrollment (years)	60 [51-67]	59 [50.2-66]	62 [53-69]	58 [49.8-66]
BMI (kg/m^2)	24.9 [22.7-27.3]	24.9 [22.7-26.9]	24.5 [22.5-26]	25.2 [23-28.7]
Leukocytes ($x10^9/L$)	7 [5.6-8.5]	7.3 [5.6-8.3]	6.9 [5.4-8.8]	7.1 [5.8-8.4]
Platelet count ($x10^9/L$)	521 [422-641]	512 [418-629]	521 [404-622]	532 [424-660]
Hematocrit (%)	41.7 [39.1-44.3]	41.4 [38.3-44.4]	42.2 [39.5-44.3]	41.4 [39.6-43.8]
JAK2 genotype:				
Wild type, n (%)	99 (40.4)	38 (44.2)	31 (39.2)	30 (37.5)
Mutated, n (%)	145 (59.2)	48 (55.8)	48 (60.8)	49 (61.3)
Not available, n (%)	1 (0.4)	0 (0)	0 (0)	1 (1.3)
CALR mutation:				
Type 1, n (%)	19 (7.8)	7 (8.1)	6 (7.7)	6 (7.5)
Type 2, n (%)	16 (6.5)	5 (5.8)	6 (7.7)	5 (6.3)
Other, n (%)	95 (38.9)	31 (36)	29 (37.2)	35 (43.8)
Not available, n (%)	115 (46.7)	43 (50)	38 (47.4)	34 (42.5)
IPSET thrombosis score, n				
0	34	14	11	9
1	41	16	9	16
2	77	23	26	28
3	45	20	13	12

4	42	11	19	12
5	5	2	1	2
6	1	0	0	1
Microvascular symptoms, n (%)	25 (10.2)	10 (11.6)	9 (11.4)	6 (7.5)
Previous thrombosis:				
MPN-related, n (%)*	10 (4.1)	3 (3.5)	2 (2.5)	5 (6.2)
Any thrombosis, n (%)	28 (11.4)	10 (11.6)	8 (10.1)	10 (12.5)
Cytoreductive therapy:				
No, n (%)	98 (40)	41 (47.7)	28 (35.4)	29 (36.2)
Yes, n (%)	147 (60)	45 (52.3)	51 (64.6)	51 (63.7)
TXB_2 before randomization, ng/ml	19 [9.3-43.2]	17.1 [8.3-32.8]	20 [11.6-56.4]	23.5 [9.8-47.8]

Quantitative values are reported as medians and [interquartile range], unless otherwise indicated.
Abbreviations: BMI: body mass index; MPN: myeloproliferative neoplasms; TX: thromboxane.
* defined as any major thrombosis occurring within 2 years before diagnosis and any time afterwards.
There were no significant differences between the randomized groups, according to the Kruskal-Wallis test or chi squared for continuous or discrete variables, respectively.

Table 2. Compliance with aspirin treatment according to pill counting in 243 evaluable ET patients.

	Fully compliant N=218	**Partially compliant N=21**	**Non compliant N=4**	**P global**
Definition	All 9 pills in the three days before V3	6-8 pills in the three days before V3	No pill in the three days before V3	
Sex:				0.23
Male, n (%)	104 (47.7)	6 (28.6)	2 (50)	
Female, n (%)	114 (52.3)	15 (71.4)	2 (50)	
Median age at enrollment (years)	60 [51.3-67]	54 [45-66]	54 [43.3-64]	0.21
Median TXB_2 at Visit 2 (ng/ml)	18.6 [8.9-42.9]	22.8 [13.6-37]	46.5 [24-107.8]	0.40
Treatment				0.60
100 od, n (%)	73 (33.5)	10 (47.6)	2 (50)	
100 bid, n (%)	71 (32.6)	7 (33.3)	1 (25)	
100 tid, n (%)	74 (33.9)	4 (19)	1 (25)	

Quantitative values are reported as medians and [interquartile range], unless otherwise indicated. Abbreviations: TX: thromboxane; P value according to the Kruskal-Wallis test or chi squared for continuous or discrete variables, respectively.

Table 3. Median values of serum TXB_2, urinary PGIM and urinary TXM before (Visit 2) and after (Visit 3) the randomized aspirin regimen in 243 evaluable ET patients.

	100 mg od N=85	100 mg bid N=79	100 mg tid N=79	P global	P bid vs.tid
$sTXB_2$ at V2 (ng/ml)	17 [8.2-33]	20 [11.6-6.4]	23.3 [9.6-46.4]	0.098	0.41
$sTXB_2$ at V3 (ng/ml)	19.3 [9.7-40]	4 [2.1-6.7]	2.5 [1.4-5.7]	<0.001	0.04
$sTXB_2$ V3/V2 ratio	1 [0.77-1.5]	0.1 [0.08-0.3]	0.1 [0.08-0.2]	<0.001	0.24
PGIM at V2 (pg/mg creatinine)	84 [50-123]	76 [47-132]	83 [53-123]	0.96	0.74
PGIM at V3 (pg/mg creatinine)	89 [54-127]	87 [46-121]	80 [47-131]	0.70	0.90
PGIM V3/V2 ratio	1.1 [0.7-1.5]	0.9 [0.7-1.4]	0.9 [0.6-1.6]	0.48	0.88
TXM at V2 (pg/mg creatinine)	485 [336-693]	641 [437-864]	515 [379-738]	0.02	0.09
TXM at V3 (pg/mg creatinine)	457 [313-674]	367 [237-541]	344 [229-487]	0.001	0.37
TXM V3/V2 ratio	0.9 [0.7-1.3]	0.7 [0.5-0.8]	0.7 [0.5-0.8]	<0.001	0.71

Data are medians and [interquartile range].

Abbreviations: TX: thromboxane; PGIM: urinary prostacyclin metabolite; TXM: urinary thromboxane metabolite; V: visit. P values refer to Spearman test (P global) and to Wilcoxon test for the bid vs tid comparison.

References

1. Patrono C, Rocca B, De Stefano V. Platelet activation and inhibition in polycythemia vera and essential thrombocythemia. *Blood*. 2013;121(10):1701-1711.
2. De Stefano V, Za T, Rossi E, et al. Recurrent thrombosis in patients with polycythemia vera and essential thrombocythemia: incidence, risk factors, and effect of treatments. *Haematologica*. 2008;93(3):372-380.
3. Rungjirajittranon T, Owattanapanich W, Ungprasert P, Siritanaratkul N, Ruchutrakool T. A systematic review and meta-analysis of the prevalence of thrombosis and bleeding at diagnosis of Philadelphia-negative myeloproliferative neoplasms. *BMC Cancer*. 2019;19(1):184.
4. Hultcrantz M, Wilkes SR, Kristinsson SY, et al. Risk and Cause of Death in Patients Diagnosed With Myeloproliferative Neoplasms in Sweden Between 1973 and 2005: A Population-Based Study. *J Clin Oncol*. 2015;33(20):2288-2295.
5. Tefferi A, Pardanani A. Essential Thrombocythemia. *N Engl J Med*. 2019;381(22):2135-2144.
6. 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.
7. Rocca B, Ciabattoni G, Tartaglione R, et al. Increased thromboxane biosynthesis in essential thrombocythemia. *Thromb Haemost*. 1995;74(5):1225-1230.
8. 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.
9. Viallard JF, Solanilla A, Gauthier B, et al. Increased soluble and platelet-associated CD40 ligand in essential thrombocythemia and reactive thrombocytosis. *Blood*. 2002;99(7):2612-2614.
10. 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.
11. 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; quiz 1387.
12. 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.
13. De Stefano V, Rossi E, Carobbio A, et al. Hydroxyurea prevents arterial and late venous thrombotic recurrences in patients with myeloproliferative neoplasms but fails in the splanchnic venous district. Pooled analysis of 1500 cases. *Blood Cancer J*. 2018;8(11):112.
14. Burch JW, Stanford N, Majerus PW. Inhibition of platelet prostaglandin synthetase by oral aspirin. *J Clin Invest*. 1978;61(2):314-319.
15. Giaretta A, Rocca B, Di Camillo B, Toffolo GM, Patrono C. In Silico Modeling of the Antiplatelet Pharmacodynamics of Low-dose Aspirin in Health and Disease. *Clin Pharmacol Ther*. 2017;102(5):823-831.
16. Dillinger JG, Sideris G, Henry P, Bal dit Sollier C, Ronez E, Drouet L. Twice daily aspirin to improve biological aspirin efficacy in patients with essential thrombocythemia. *Thromb Res*. 2012;129(1):91-94.
17. 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.
18. Davi G, Patrono C. Platelet activation and atherothrombosis. *N Engl J Med*. 2007;357(24):2482-2494.
19. Coxib, traditional NTC, Bhala N, 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.
20. 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.
21. FitzGerald GA, Brash AR, Oates JA, Pedersen AK. Endogenous prostacyclin biosynthesis and platelet function during selective inhibition of thromboxane synthase in man. *J Clin Invest*. 1983;72(4):1336-1343.
22. Cavalca V, Rocca B, Squellerio I, et al. In vivo prostacyclin biosynthesis and effects of different aspirin regimens in patients with essential thrombocythaemia. *Thromb Haemost*. 2014;112(1):118-127.

23. 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.
24. 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.
25. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003340.pdf.
26. FitzGerald GA, Oates JA, Hawiger J, et al. Endogenous biosynthesis of prostacyclin and thromboxane and platelet function during chronic administration of aspirin in man. *J Clin Invest*. 1983;71(3):676-688.
27. Ciabattoni G, Pugliese F, Davi G, Pierucci A, Simonetti BM, Patrono C. Fractional conversion of thromboxane B2 to urinary 11-dehydrothromboxane B2 in man. *Biochim Biophys Acta*. 1989;992(1):66-70.
28. 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.
29. 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.
30. Geyer HL, Kosiorek H, Dueck AC, et al. Associations between gender, disease features and symptom burden in patients with myeloproliferative neoplasms: an analysis by the MPN QOL International Working Group. *Haematologica*. 2017;102(1):85-93.
31. 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.
32. 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; quiz 5252.
33. Petrucci G, Rizzi A, Cavalca V, et al. Patient-independent variables affecting the assessment of aspirin responsiveness by serum thromboxane measurement. *Thrombosis and Haemostasis*. 2016;116(5):891-896.
34. Rocca B, Santilli F, Pitocco D, et al. The recovery of platelet cyclooxygenase activity explains interindividual variability in responsiveness to low-dose aspirin in patients with and without diabetes. *J Thromb Haemost*. 2012;10(7):1220-1230.
35. FitzGerald GA, Brash AR, Falardeau P, Oates JA. Estimated rate of prostacyclin secretion into the circulation of normal man. *J Clin Invest*. 1981;68(5):1272-1276.
36. 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.
37. Pagliaccia F, Habib A, Pitocco D, et al. Stability of urinary thromboxane A2 metabolites and adaptation of the extraction method to small urine volume. *Clin Lab*. 2014;60(1):105-111.
38. Team RDC. A language and environment for statistical computing (v 3.6.1). *R Foundation for Statistical Computing, Vienna*. 2019.
39. Foster EDA. Open Science Framework (OSF). *Journal of the Medical Library Association*. 2017;105(2):206.
40. Petrucci G, Zaccardi F, Giaretta A, et al. Obesity is associated with impaired responsiveness to once-daily low-dose aspirin and in vivo platelet activation. *J Thromb Haemost*. 2019;17(6):885-895.
41. 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.
42. Patrignani P, Tacconelli S, Piazuelo E, et al. Reappraisal of the clinical pharmacology of low-dose aspirin by comparing novel direct and traditional indirect biomarkers of drug action. *J Thromb Haemost*. 2014;12(8):1320-1330.

43. Patrignani P, Filabozzi P, Patrono C. Selective cumulative inhibition of platelet thromboxane production by low-dose aspirin in healthy subjects. *J Clin Invest*. 1982;69(6):1366-1372.
44. Patrono C, Baigent C. Role of aspirin in primary prevention of cardiovascular disease. *Nat Rev Cardiol*. 2019;16(11):675-686.
45. Patrono C, Morais J, Baigent C, et al. Antiplatelet Agents for the Treatment and Prevention of Coronary Atherothrombosis. *J Am Coll Cardiol*. 2017;70(14):1760-1776.
46. 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.
47. Capone ML, Tacconelli S, Sciulli MG, et al. Clinical pharmacology of platelet, monocyte, and vascular cyclooxygenase inhibition by naproxen and low-dose aspirin in healthy subjects. *Circulation*. 2004;109(12):1468-1471.
48. Patrono C, Baigent C. Nonsteroidal anti-inflammatory drugs and the heart. *Circulation*. 2014;129(8):907-916.

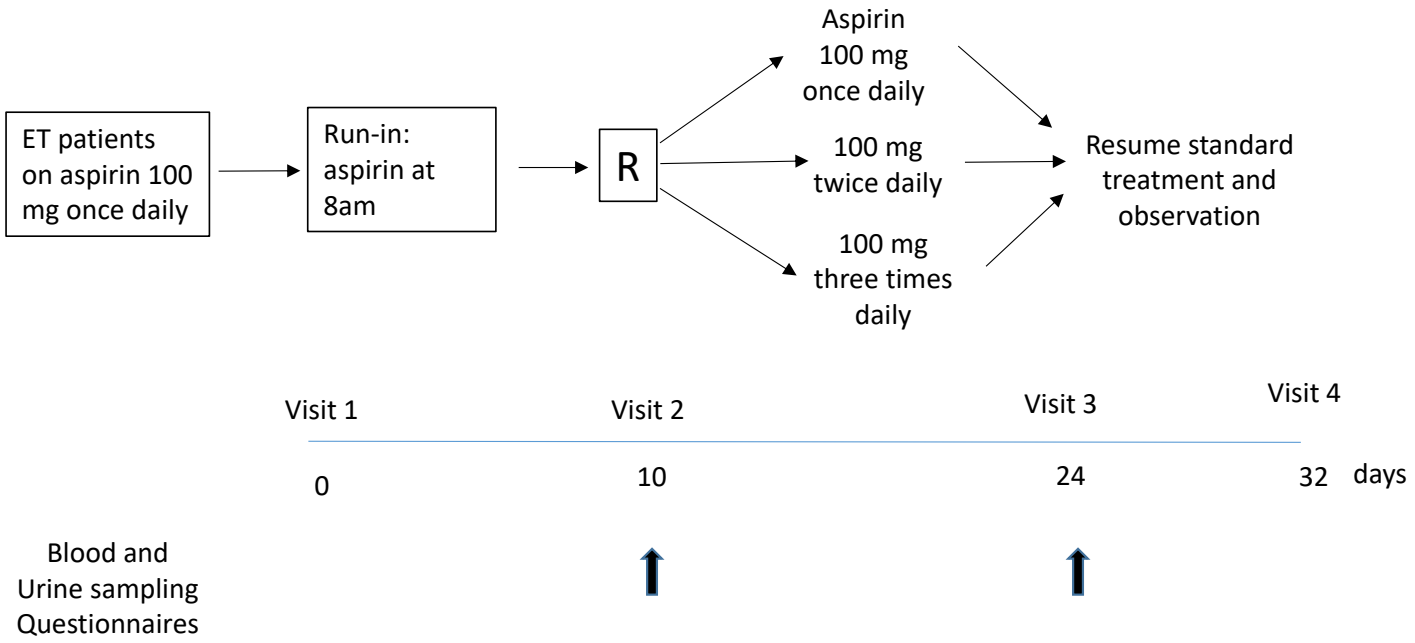

Figure 1

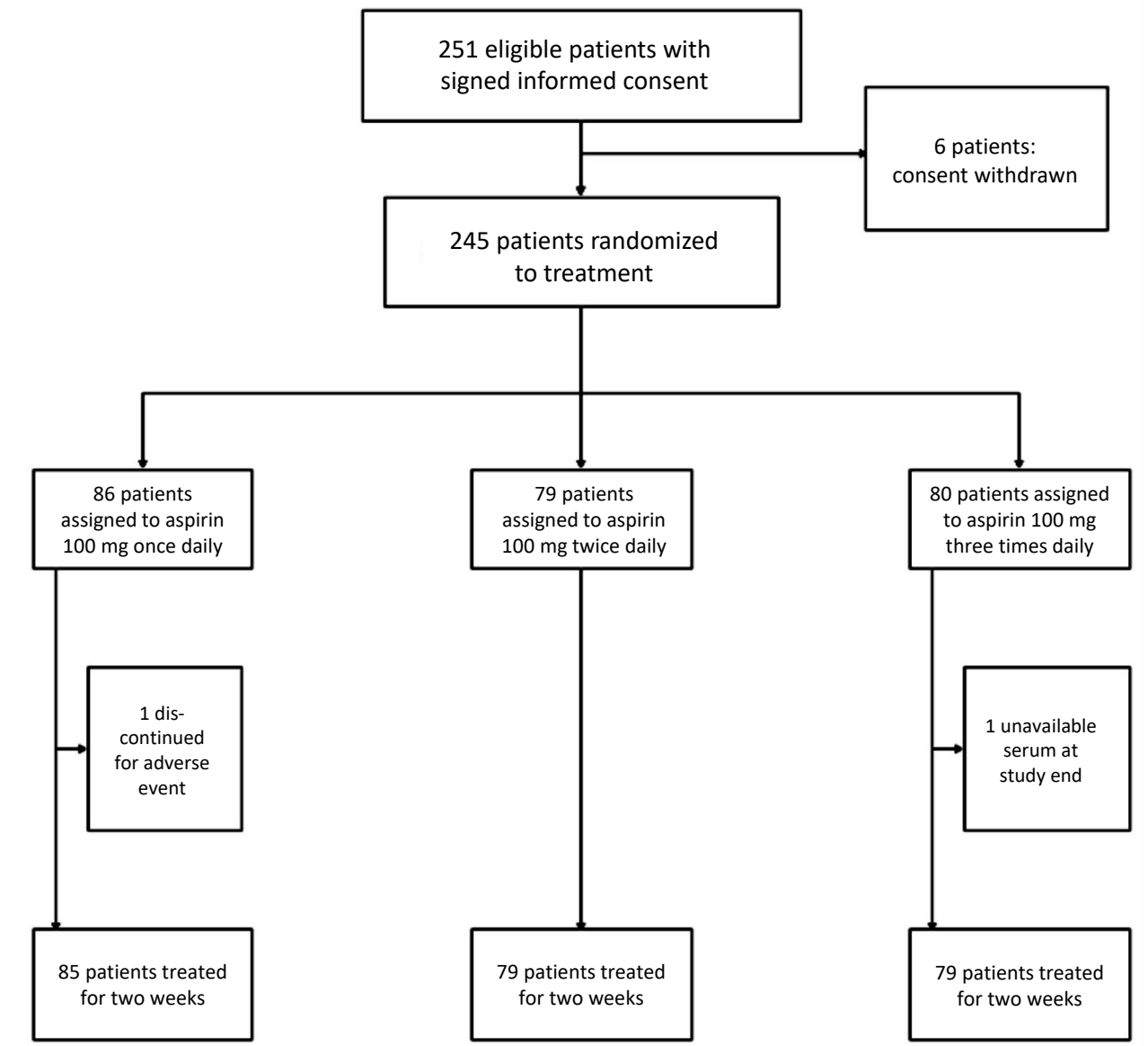

Figure 2

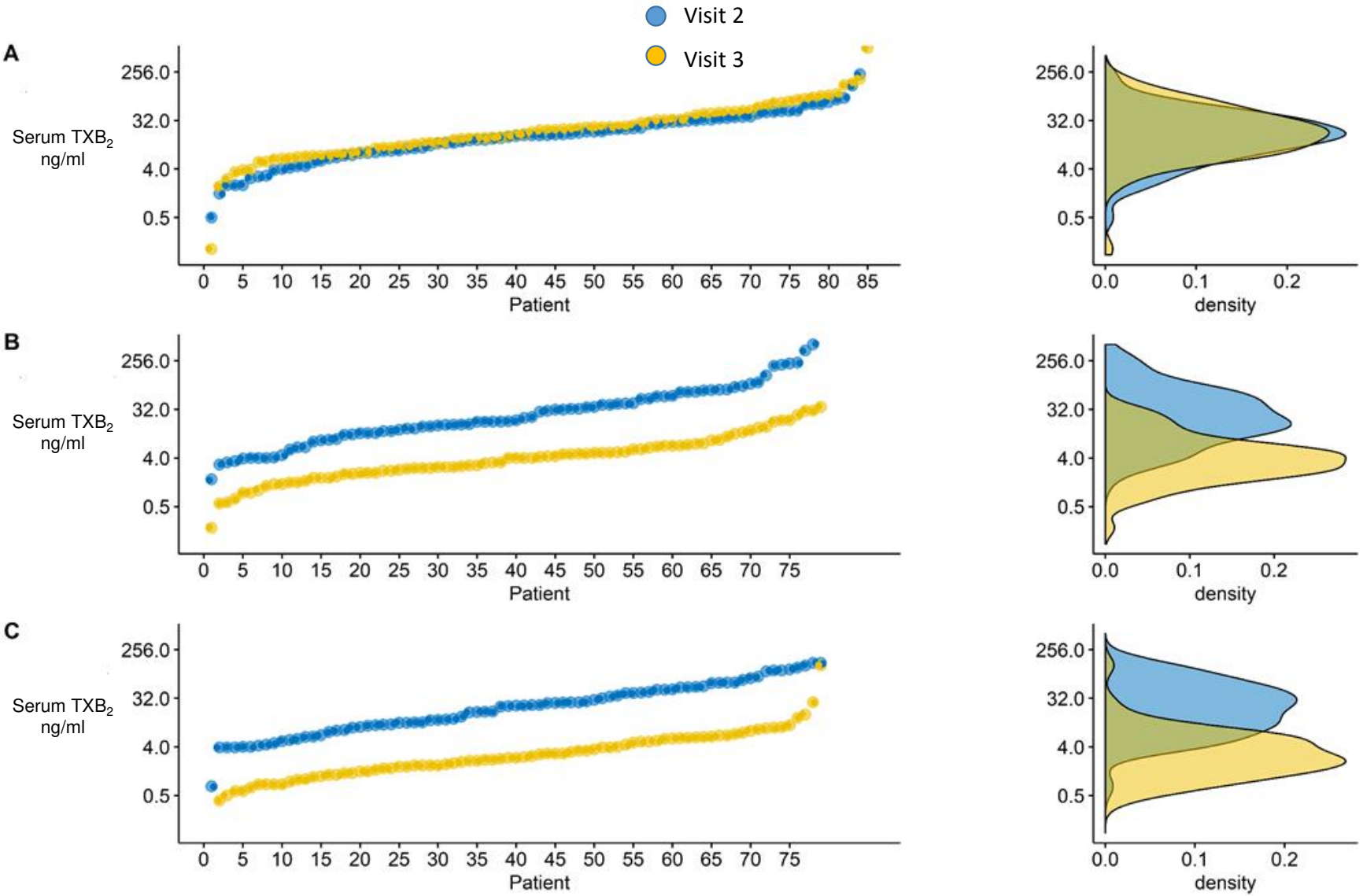

Figure 3

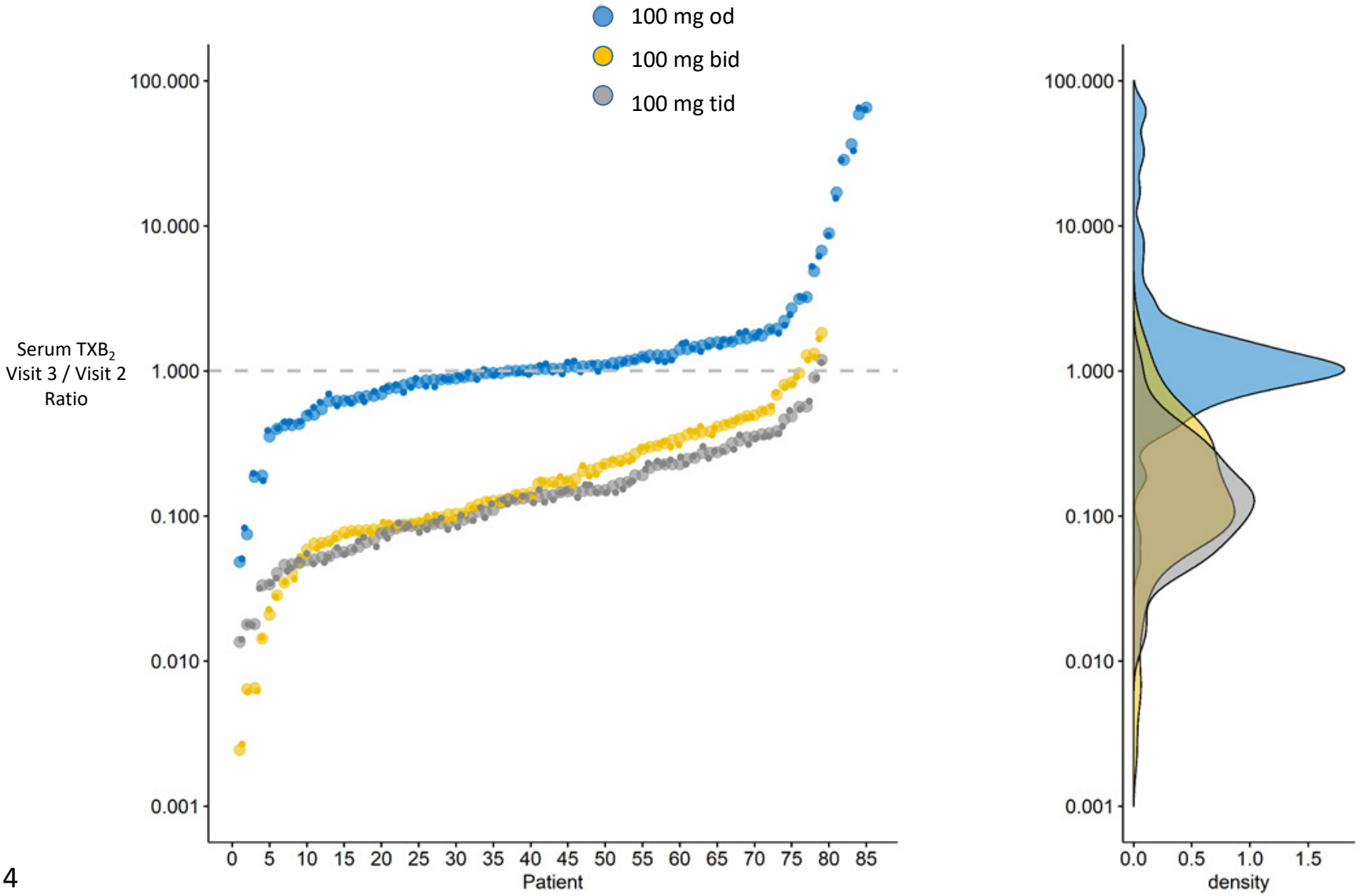

Figure 4

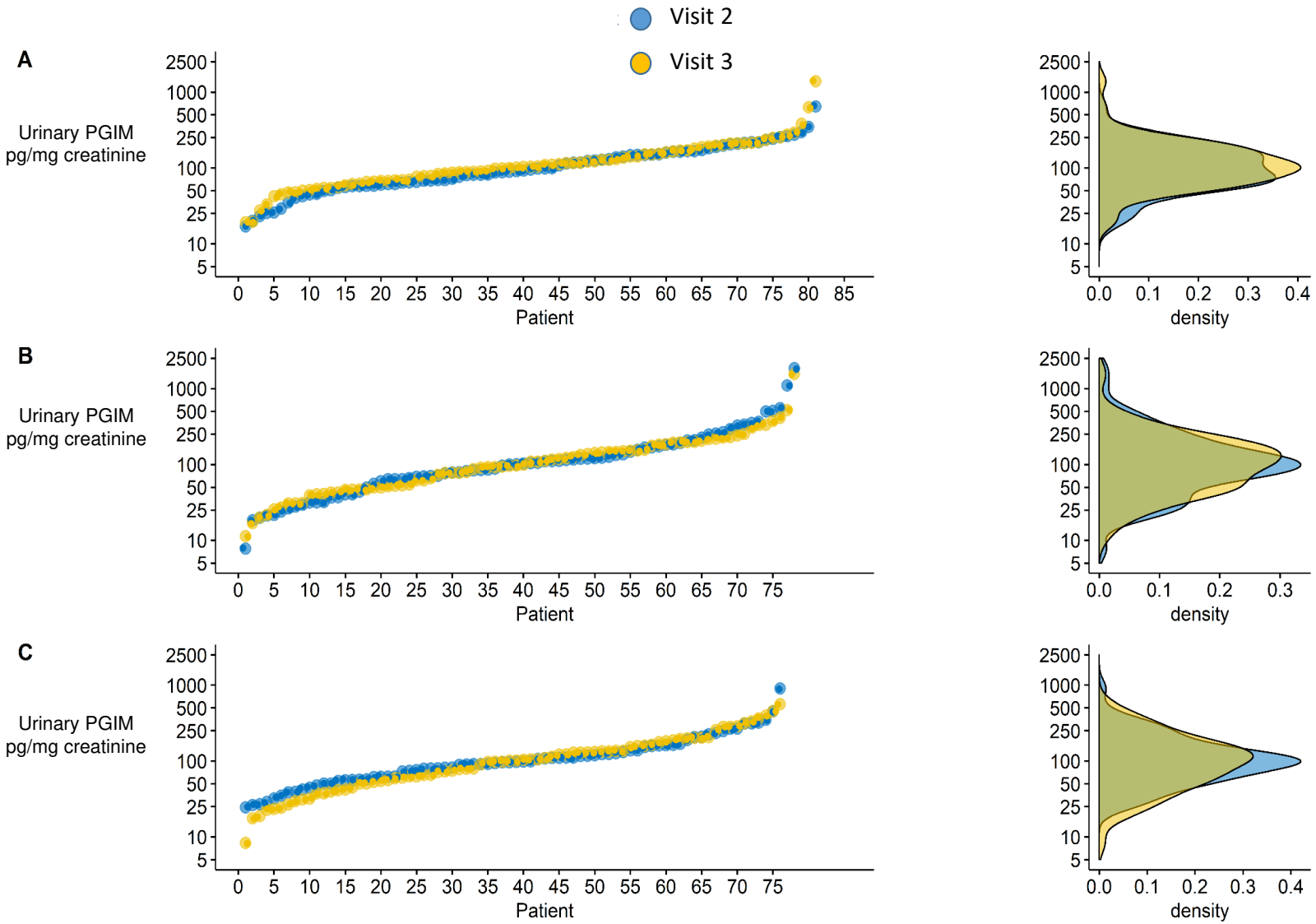

Figure 5

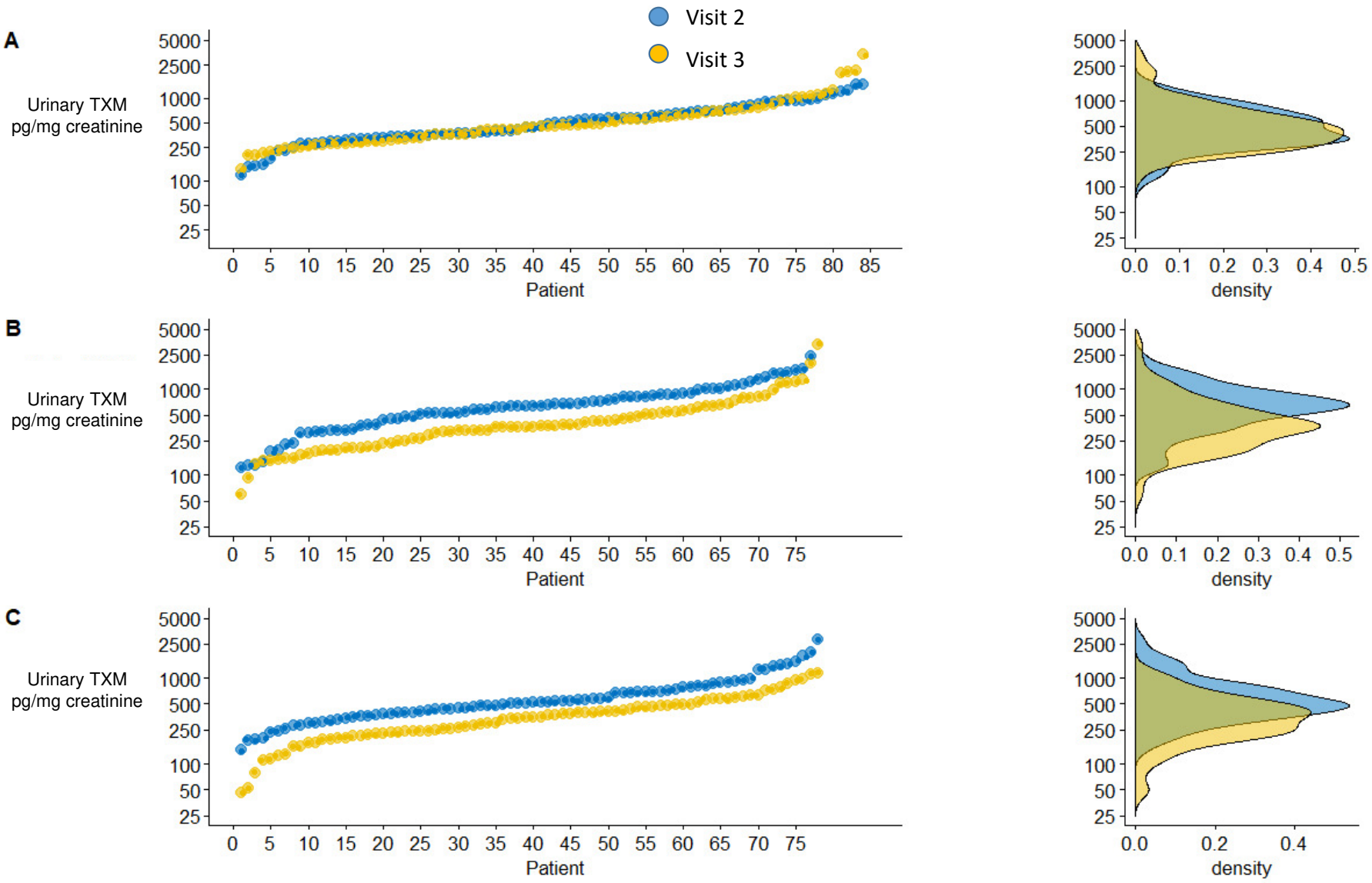

Figure (

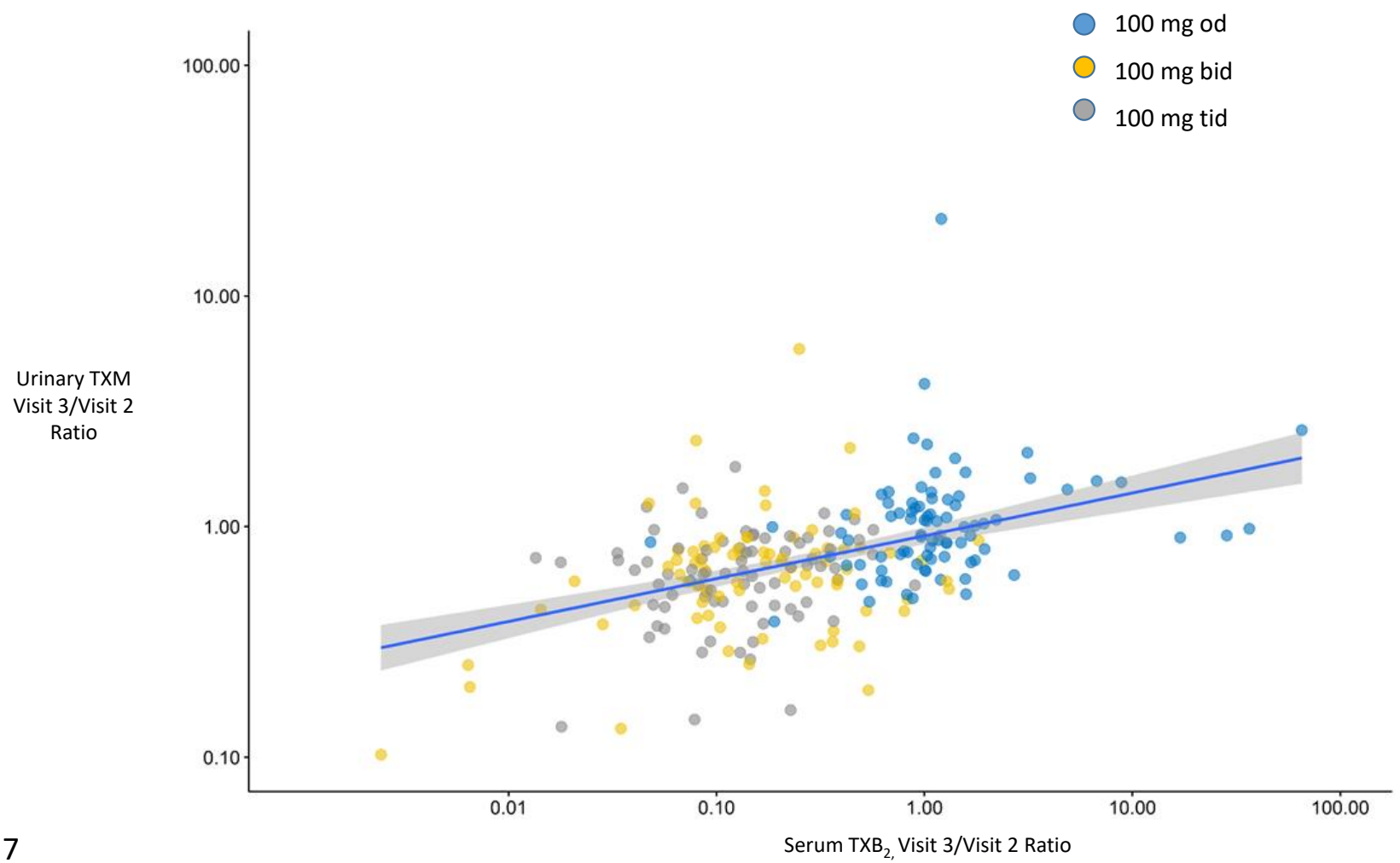

Figure 7