Effect of Ticagrelor Versus Clopidogrel on Aortic Stiffness in Patients With Coronary Artery Disease

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Background—We compared the acute and midterm effect of ticagrelor versus clopidogrel on aortic stiffness.

Methods and Results—We studied 117 patients in a randomized, assessor-blinded, parallel-group trial. The acute effect of ticagrelor was studied in 58 patients randomized (1:1) to receive a loading dose of clopidogrel (600 mg) or ticagrelor (180 mg). Carotid-femoral pulse wave velocity (cfPWV) was measured before, 3, and 24 hours after the loading dose. The midterm effect (30-day treatment period) was studied in 59 subjects who underwent percutaneous coronary intervention and were randomized to either clopidogrel (75 mg, OD) or ticagrelor (90 mg BID). cfPWV was measured before and at 30 days of treatment. Circulating markers of inflammation and endothelial function were measured at all study points. Repeated-measures analysis showed a significant main effect for treatment (P=0.03), with the ticagrelor showing a reduction in cfPWV after treatment. cfPWV at 24 hours was significantly lower in the ticagrelor group compared with the clopidogrel group (P=0.017) (maximal response reduction by 0.42±0.26 m/s). At 30 days, cfPWV decreased in the ticagrelor group, whereas there was no change with clopidogrel (−0.43±0.57 versus 0.12±0.14 m/s, P=0.004). There were no significant changes in both the acute and midterm study period in the pro-inflammatory and endothelial function parameters.

Conclusions—URL: https://www.clinicaltrials.gov. Unique identifier: NCT02071212. Ticagrelor decreases cfPWV for 24 hours after the loading dose and at 1 month post–percutaneous coronary intervention compared with clopidogrel. Considering that aortic stiffness is an independent predictor of cardiovascular events, this finding may have clinical implications regarding the beneficial effect of ticagrelor.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT02071212. (***J Am Heart Assoc***. 2019;8: e012521. DOI: 10.1161/JAHA.119.012521.)

Key Words: adenosine • aortic stiffness • clopidogrel • coronary artery disease • ticagrelor

Aortic stiffness is an independent predictor of cardiovascular events and mortality in the elderly, hypertensive, diabetics, and patients with chronic renal failure, as well as in the general population.[1–4] Arterial stiffness is influenced by several factors that regulate not only the static components associated with the architecture and composition of the arterial wall but also the dynamic properties of the arterial vessels that are related to vascular tone.[5] Although structural changes in arteries constitute a major factor in the age-related increase in arterial stiffness, several lines of evidence suggest that the endothelium may play an important role in the local functional regulation of stiffness by releasing vasoactive substances, such as nitric oxide.[6]

Ticagrelor, an oral, direct-acting, reversibly binding P2Y12 receptor antagonist, reduced cardiovascular events and all-cause mortality as compared with those treated with clopidogrel in the PLATO (Platelet Inhibition and Clinical Outcomes) trial[7]. It is recommended by both European Society of Cardiology and American College of Cardiology guidelines as first-line treatment for the prevention of atherothrombotic

Clinical Perspective

What Is New?

- Considering that aortic stiffness is regulated by vascular tone and that ticagrelor induces vasorelaxation through its adenosine-like effect and potentially via endothelial function improvement, we hypothesized that administration of ticagrelor would improve aortic stiffness.
- The study compared for the first time the acute (24 hours) and midterm (30 days) effect of ticagrelor versus clopidogrel on aortic stiffness in patients with indication for elective angiography and ad hoc or elective percutaneous coronary intervention.
- Circulating markers of inflammation and endothelial function were measured at all study points.

What Are the Clinical Implications?

- The study shows that ticagrelor, in contrast to clopidogrel, improves aortic stiffness during the first 24 hours after a loading dose and at 1 month after continuous administration in patients undergoing elective percutaneous coronary intervention.
- The more potent acute and midterm favorable effect of ticagrelor compared with clopidogrel on aortic elastic properties imply pleiotropic effects beyond P2Y12 inhibition that may be a contributory factor in cardiovascular risk reduction reported in patients receiving this P2Y12 platelet receptor antagonist.

events in adults with acute coronary syndromes (ACS) and patients with coronary artery disease (CAD) who undergo percutaneous coronary intervention (PCI).[8–10] Although this effect may be attributed to the potent antiplatelet action of ticagrelor, a palette of advantageous pleiotropic effects associated with the increased concentration of adenosine has also been proposed.[11–14] The pleiotropic effects of ticagrelor may include restoration of myocardial injury after an ischemic event,[15] promotion of the release of anticoagulation factors,[16–18] and anti-inflammatory effects.[19]

Recent data indicate that ticagrelor may affect vascular function. Indeed, while there are conflicting results,[20,21] most of the studies comparing either prasugrel or clopidogrel to ticagrelor support a beneficial impact of the latter on vascular and circulating markers of endothelial function, which seems to be significant in the higher-risk patients, such as patients with ACS and diabetes mellitus requiring PCI,[19] previous ACS,[22] stable CAD,[23] successful chronic total occlusion recanalization,[24] and chronic obstructive pulmonary disease.[25] Considering that aortic stiffness is regulated by vascular tone and that ticagrelor induces vasorelaxation through its adenosine-like effect and potentially via endothelial function improvement, we

hypothesized that administration of ticagrelor would improve aortic stiffness. Therefore, we performed an assessor-blinded, randomized, active controlled, parallel-group trial in order to compare the acute (24 hours) and midterm (30 days) effect of ticagrelor versus clopidogrel on aortic stiffness.

Methods

The data and analytic methods can be made available to other researchers for purposes of reproducing the results or replicating the procedure pending justified request. Researchers can contract us by e-mail.

Study Population

Male and female patients 18 to 79 years of age were eligible if they had indication (1) for elective coronary angiography (angina, positive stress test/single-photon emission computed tomography/stress echo) for inclusion in the "acute" study period, and (2) for either ad hoc or elective PCI for inclusion in the "midterm" study period. A total of 129 patients were screened for inclusion at First Department of Cardiology from February 2014 to November 2017 (flow chart, Figure 1). Exclusion criteria are provided in Table S1. The 2 study populations have some patients in common. Specifically, 6 patients were participants of the acute study who were eligible for PCI intervention and continued in the midterm study (ticagrelor, n=3; clopidogrel, n=3).

In the *acute (24-hour) study*, a total of 64 patients were screened for eligibility. Of them, 3 patients refused to participate and 1 patient was excluded. Therefore, a total of 60 patients were randomized (ticagrelor, n=30; clopidogrel, n=30). One patient at each treatment arm was withdrawn from the study because they had not angiographically documented CAD and thus they were not eligible for completing the 24-hour administration of the drugs. Finally, 58 patients with significant (>50%) stenosis of the epicardial coronary arteries (ticagrelor, n=29; clopidogrel, n=29) completed the acute study.

In the *midterm (30-day) study*, a total of 65 patients were screened for eligibility. Of them, 2 patients refused to participate and 1 patient was excluded. Therefore, a total of 62 patients were randomized (ticagrelor, n=32; clopidogrel, n=30). One patient in the ticagrelor group and 2 patients in the clopidogrel group were lost to follow-up. Finally, 59 patients (ticagrelor, n=31; clopidogrel, n=28) completed the 30-day study period.

All participants provided informed consent before any study specific procedures. The study was performed in accordance with ethical principles that have their origin in the Declaration of Helsinki (2008) and are consistent with International Conference on Harmonization (ICH)/Good Clinical Practice (GCP), and applicable local regulatory requirements.
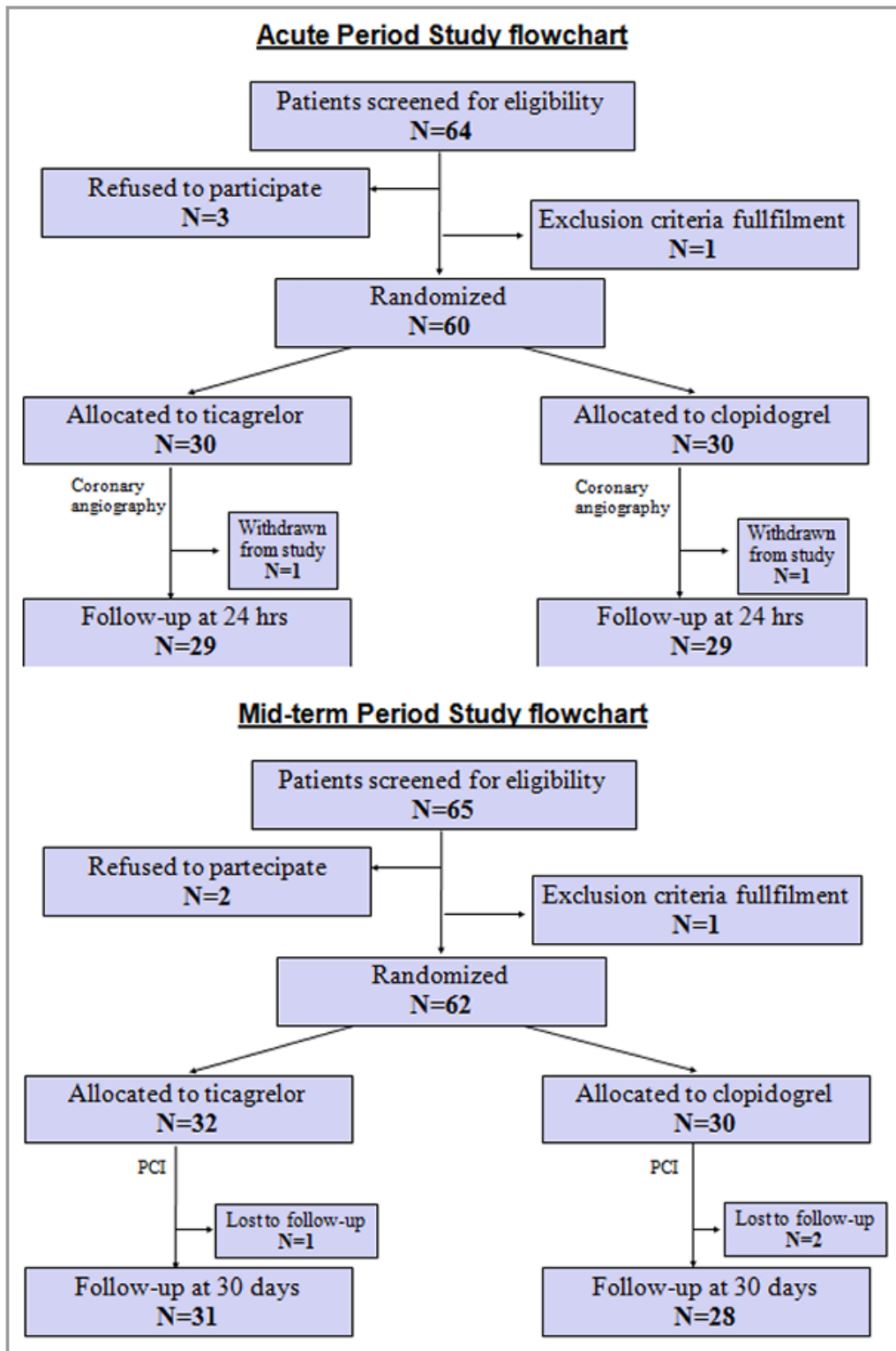
Acute Period Study flowchart

Patients screened for eligibility
N=64

Refused to participate
N=3

Exclusion criteria fullfilment
N=1

Randomized
N=60

Allocated to ticagrelor
N=30

Coronary angiography

Withdrawn from study
N=1

Follow-up at 24 hrs
N=29

Allocated to clopidogrel
N=30

Coronary angiography

Withdrawn from study
N=1

Follow-up at 24 hrs
N=29

Mid-term Period Study flowchart

Patients screened for eligibility
N=65

Refused to partecipate
N=2

Exclusion criteria fullfilment
N=1

Randomized
N=62

Allocated to ticagrelor
N=32

PCI

Lost to follow-up
N=1

Follow-up at 30 days
N=31

Allocated to clopidogrel
N=30

PCI

Lost to follow-up
N=2

Follow-up at 30 days
N=28

Figure 1. Participant recruitment flow chart of the acute and midterm studies.

Study Design

This was a single-center, NOVELTY study (Randomized, assessor-blinded, active controlled, parallel-group trial to compare ticagrelor versus clopidogrel on the reduction of arterial stiffness and wave reflections in patients with coronary artery disease) (*NCT number:* NCT02071212). The study consists of 2 study periods: *24-hour acute period:* The acute effect of ticagrelor was

studied in 58 subjects with an indication for coronary angiography randomized (1:1) to receive a loading dose of clopidogrel (600 mg) or ticagrelor (180 mg) before angiography. Carotid-femoral pulse wave velocity (cfPWV) was measured as an index of aortic stiffness before (baseline), 3 and 24 hours after the loading dose of each regimen. *Midterm, 30-day period:* Fifty-nine subjects who underwent PCI were randomized to either the clopidogrel (n=28) or ticagrelor study arm (n=31). Part of this "midterm" period population consists of 3 subjects who were included in the "acute" period (n=1 clopidogrel and n=2 ticagrelor) and proceeded ad hoc to PCI. cfPWV was measured before (baseline) and at 30 days treatment with either clopidogrel (75 mg OD) or ticagrelor (90 mg BID) after PCI. Since patients were not blinded and there was a possibility of revealing their treatment to the outcome assessor, all possible efforts were made to overcome this risk by encouraging patients not to reveal to outcome assessors the treatment they received. The outcome assessor (CG) is trained and qualified to perform the measurements, and he was blinded to both treatment allocation and study purpose and hypothesis.

All participants in both acute and midterm studies were requested to fast for at least 8 hours before each study visit and abstain from caffeine and nicotine for at least 8 hours before each session. To avoid circadian-related blood pressure (BP) differences, all vascular studies were performed in the morning between 8 and 10 AM, in a quiet, temperature-controlled room at 23°C. Following a 15-minute rest, brachial BP measurements were taken 3 times using an oscillometric device (Omron M4-I, CE 0197; Hoofddorp, The Netherlands). Subsequently, aortic stiffness measurements were obtained.

Evaluation of Aortic Stiffness

cf-PWV, an established index of aortic stiffness, was calculated from measurements of pulse transit time and the distance traveled between 2 recording sites (PWV equals distance in meters divided by transit time in seconds) with a validated noninvasive device (Complior; Artech Medical, Paris, France), which allows online pulse wave recording and automatic calculation of PWV. Two different pulse waves were obtained simultaneously at 2 sites (at the base of the neck for the common carotid and over the right femoral artery) with 2 transducers. Distance is defined as the distance from the suprasternic notch to femoral artery minus the distance from the carotid artery to the suprasternic notch.[5] Brachial blood pressure was measured and entered into the Complior Analyse software, and then signal acquisition was launched.

Evaluation of Inflammatory and Endothelial Markers

Immediately after acquisition of venous blood, plasma or serum was separated by centrifugation for 15 minutes, then placed in aliquots and stored at −70°C for the measurement of inflammatory/endothelial markers. Tumor necrosis factor-α (TNF-α), endothelin-1, and ADAMTS-13 (adisintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), also known as von-Willebrand factor (vWF)–cleaving protease, were measured using a 4.5-hour solid-phase ELISA (R&D Systems, Minneapolis, MN). By cleaving vWF, ADAMTS-13 downregulates not only thrombosis but also inflammation.[26] As a result, decreased ADAMTS-13 activity accelerates inflammatory diseases and is associated with acute and chronic inflammation.[27] The measurements of circulating markers/mediators were made by researchers who were unaware of the study hypothesis.

Statistical Analysis

Data are expressed as mean±SD or median (interquartile range) for continuous variables. Data for categorical variables were expressed as number and percentage of patients. Chi-square test was used for categorical variables; Student *t* tests for normal distribution and Kruskal–Wallis tests for nonnormal distribution were used for continuous variables.

24-Hour acute period

A 2-way ANCOVA method was initially applied followed by testing simple main effects if the homogeneity of variance parametric assumption was not violated (ie, multiple comparisons of different levels of each factor that were Bonferroni-corrected if the factor had 2 or more levels) in case of main-factor or interaction significance. Our primary test that was used for the sample size calculation was the difference between groups in cfPWV at 3 and 24 hours. Specifically, we applied a correction and a decrease in the $P=0.025$ for the comparison of cfPWV in the 2 different time periods (3 and 24 hours). The interaction and the main effects are presented. In cases in which ANCOVA yielded a nonsignificant interaction, multiple comparisons with Tukey Honestly Significant Difference post hoc was done for the different treatment time points. Comparisons of respective regimen on each end point and the same regimen on different end points were obtained from the model and compared by 1-way ANOVA. Finally, additional analysis was conducted to assess the impact of the 2 different treatments (ticagrelor or clopidogrel) on cfPWV changes across 3 time points (baseline, 3, and 24 hours) using mean arterial pressure as a covariate.

Midterm, 30-day period

Changes from baseline were calculated as the value at the end of treatment (over the 1-month period) subtracted from the value at the beginning. Comparisons before and after treatment were analyzed by paired Student *t* test. A mixed

between-within subjects ANCOVA was conducted to assess the overall difference between treatments on cfPWV across the 2 time points, using baseline levels as covariate. Additional analysis after adjusting for mean arterial pressure was performed to examine the influence of BP on cfPW changes throughout the study period.

Determination of sample size

Sample size calculations were based on data from previous studies in our unit,[28] which showed that the SD of cfPWV with characteristics similar to those of our study population was 1.1 m/s and the short-term (60 minutes) effect of treatment (vardenafil) on aortic stiffness was 0.7 m/s. Therefore, we estimated that treatment with ticagrelor would be associated with a minimum clinically meaningful difference at 3 and 24 hours in cfPWV (the measure of arterial stiffness as the primary end point of our study) of ≈0.6 to 0.9 of 1.0 SD of this parameter in a parallel-design study, which is the case for the present study, and therefore a reduction of that magnitude would constitute a significant clinical change for our primary end point. We finally concluded that 0.75 of 1 SD would be a reasonable estimate for power size calculation. The proper Bonferroni correction was applied ($P=0.05/2=0.025$). Therefore, we estimated that a minimum of 29 patients treated with ticagrelor and 29 patients treated with clopidogrel would provide 80% power (Data S1). Also, in a post hoc sample size calculation group, sample sizes of 27 and 27 achieve 81% power to detect a difference of 0.75 in a design with 3 repeated measurements having a Compound Symmetry covariance structure when the SD is 1, the correlation between observations on the same subject is 0.900, and the α level is 0.05. However, because of the short-term study period, a dropout rate of <5% is expected, and therefore 30 patients in each arm were considered adequate for the evaluation of the study primary outcome variable.

A $P<0.05$ was considered statistically significant. Analyses were performed using SPSS for Windows (version 18.0, SPSS Inc., Chicago, IL).

Results

Patient Characteristics

Key patient baseline characteristics and nonstudy cardiovascular medications of both acute and midterm study are summarized in Table 1. The 2 treatment groups were well balanced with regard to all baseline clinical and biochemical parameters. There were no major bleeding complications; minor bleeding occurred in 2 patients (clopidogrel, $n=1$; ticagrelor, $n=1$). In the midterm study, 4 patients presented with dyspnea that resolved spontaneously within 48 hours not leading to treatment discontinuation and study withdrawal.

Acute Study

Peripheral BP and heart rate

Table 2 shows BP and heart rate after ticagrelor and clopidogrel loading dose. Analysis of BP levels at the different time points showed no significant change of BP and heart rate at 3 and 24 hours with ticagrelor compared with clopidogrel (all $P>0.05$ by 2-way ANOVA, Table 2).

Carotid-femoral PWV

Intragroup comparisons in the ticagrelor group showed a significant decrease in cfPWV levels over time (1-way ANOVA, $P=0.02$). cfPWV at 24 hours was reduced compared with cfPWV at baseline (maximal response reduction by 0.45 ± 0.31 m/s, $P<0.01$) and 3 hours (by 0.34 ± 0.21 m/s, $P=0.03$), respectively, whereas there were no significant differences between baseline and 3 hours ($P=0.34$). Intragroup comparisons in the clopidogrel group showed no significant changes in cfPWV over time (1-way ANOVA, $P=0.32$); and there were no intragroup differences between time points (baseline versus 3 hours, $P=0.24$; baseline versus 24 hours, $P=0.76$ and 3 versus 24 hours, $P=0.20$).

A between–within subjects ANCOVA was conducted to assess the impact of the 2 different treatments (clopidogrel, ticagrelor) on participants' aortic stiffness, across 3 time periods (pre-intervention, and 3- and 24-hour follow-up). There was no significant interaction between treatment type and time ($F=2.03$, $P=0.27$). There was a statistically significant main effect for treatment ($F=4.16$, 0.03, Figure 2), with the ticagrelor group showing a reduction in aortic stiffness after treatment. Post hoc comparisons with Tukey Honestly Significant Difference test showed that there was no significant difference in cfPWV at baseline or at 3 hours ($P=0.15$), whereas the cfPWV at 24 hours was significantly lower in the ticagrelor group compared with the clopidogrel group ($P=0.017<0.025$, Figure 2), (maximal response reduction by $0.42\pm0.26/m/s$) suggesting a difference in the effectiveness of the 2 regimens on aortic stiffness at 24 hours even after applying a Bonferroni correction. The main effect comparing the 3 time periods was not significant ($F=0.42$, $P=0.55$), suggesting no significant changes in aortic stiffness during the 3 time periods. The main effect for treatment remained statistically significant even after adjustment for mean arterial pressure ($F=4.09$, $P=0.045$).

Inflammatory and endothelial markers

Baseline TNF-α and ADAMTS-13 (vWF) were significantly higher in the ticagrelor group compared with the clopidogrel group ($P=0.02$ and $P<0.001$, respectively), whereas endothelin-1 levels were similar between the 2 study groups (Table 1). At the 24-hour time point, there was a nonsignificant reduction of

Table 1. Baseline Characteristics of the Patients According to Treatment Group

	Acute Study			Midterm Study		
	Ticagrelor	Clopidogrel	P Value	Ticagrelor	Clopidogrel	P Value
Age, y	64±10	63±11	0.42	63±8	62±8	0.44
Male, n (%)	20 (67)	18 (60)	0.36	22 (69)	25 (83)	0.25
BMI, kg/m^2	27±2	28±4	0.36	28±3	28±3	0.76
Risk factors, n (%)						
Smokers	13 (43)	12 (40)	0.28	11 (34)	16 (53)	0.10
Hypertension	23 (77)	21 (70)	0.42	25 (78)	26 (87)	0.64
Diabetes mellitus	7 (23)	6 (20)	0.52	7 (22)	5 (17)	0.24
Dyslipidemia	21 (70)	20 (67)	0.48	23 (72)	24 (80)	0.37
Biochemical parameters						
Total cholesterol, mg/dL	206±21	208±24	0.91	206±19	200±19	0.21
HDL-C, mg/dL	44±5	43±4	0.80	44±5	44±4	0.92
Triglycerides, mg/dL	131 (92–175)	133 (89–183)	0.91	123 (78–154)	119 (75–146)	0.43
LDL-C, mg/dL	130±16	132±19	0.83	158±14	143±14	0.18
Creatinine, mg/dL	1.0±0.2	1.1±0.3	0.85	1.1±0.2	1.1±0.2	0.92
Inflammatory and endothelial markers						
TNF-α, pg/mL	10.2 (6.8–11.7)	8.8 (5.7–10.6)	0.02	11.7 (7.5–14.2)	10.4 (7.1–11.8)	<0.001
ADAMTS-13, ng/mL	862 (612–1036)	683 (486–883)	<0.001	856 (572–1124)	730 (413–956)	<0.001
Endothelin-1, pg/mL	4.8±1.6	4.5±1.7	0.72	4.7±1.4	4.8±1.4	0.90
cfPWV, m/s	8.3±1.2	8.3±1.8	0.95	9.6±1.6	9.1±1.3	0.19
Cardiovascular disease drugs (n, %)						
β-Blockers	15 (50)	10 (33)	0.26	12 (38)	8 (27)	0.18
CCB	8 (27)	7 (23)	0.45	9 (28)	8 (27)	0.46
ACEi	10 (33)	8 (27)	0.23	9 (28)	8 (27)	0.46
ARBs	13 (43)	14 (47)	0.49	16 (50)	14 (47)	0.32
Statins	21 (70)	20 (67)	0.48	28 (88)	24 (80)	0.11
Angiographic findings (n,%)						
1-VD	11 (37)	13 (43)	0.25	10 (31)	11 (37)	0.28
2- or 3-VD	18 (60)	16 (53)	0.43	22 (69)	19 (63)	0.30
MI	8 (27)	10 (33)	0.45	5 (17)	5 (13)	0.32
PAD	1 (3)	2 (7)	0.12	1 (3)	3 (10)	0.05

Values are mean±SD, n (%) or median (interquartile range). ACEi indicates angiotensin receptor enzyme inhibitors; ADAMTS-13, adisintegrinand metalloproteinase with thrombospondin type 1 motif 13; ARBs, angiotensin receptor blockers; BMI, body mass index; CCB, calcium channel blockers; cfPWV, carotid-femoral pulse wave velocity; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; PAD, peripheral artery disease; TNF-α, tumor necrosis factor; VD, vessel disease.

TNF-α levels in the ticagrelor group (by 0.34 pg/mL, P=0.47) and a nonsignificant increase in the clopidogrel group (by 0.24 pg/mL, P=0.36) in comparison with baseline. There were no statistically significant differences between time points in both study groups. Changes of ADAMTS-13 (vWF) levels, 24 hours after either ticagrelor or clopidogrel loading dose, were minimal (P=0.36 and P=0.26, respectively). Endothelin-1 increased in both the clopidogrel group and the ticagrelor group

at the 24-hour time point; however, the increase after clopidogrel loading dose was greater (by 0.1 pg/mL, P=0.06) (Figure S1).

Midterm Study

Peripheral BP and heart rate

Table 3 shows changes in BP and heart rate before and 30 days after ticagrelor or clopidogrel therapy. Baseline

Table 2. Effect of Ticagrelor Versus Clopidogrel on BP and Heart Rate (Acute Study)

	Ticagrelor			Clopidogrel			
	Base	3 Hours	24 Hours	Base	3 Hours	24 Hours	P Value*
Systolic BP, mm Hg	133±14	130±25	127±5	132±16	132±12	127±12	0.33
Diastolic BP, mm Hg	78±6	74±11	76±13	78±7	78±7	77±7	0.59
Pulse pressure, mm Hg	53±17	55±18	49±5	54±14	54±13	50±12	0.54
Mean BP, mm Hg	93±19	91±18	90±18	93±20	93±17	90±19	0.62
Heart rate, bpm	61±9	63±10	67±7	65±11	66±11	69±11	0.47
cfPWV, m/s	8.3±2.1	8.3±2.0	7.8±2.0	8.3±1.8	8.6±2.1	8.3±1.8	0.02

BP indicates blood pressure; cfPWV, carotid-femoral pulse wave velocity.
*P values by 2-way ANOVA.

peripheral BP and heart rate measurements were not different between the 2 treatment arms. Although patients treated with clopidogrel had a mild increase in systolic and diastolic BP and heart rate after 30 days compared with patients treated with ticagrelor, the detected differences from baseline were not statistically significant (all $P>0.05$, by 2-way ANOVA, Table 3).

Carotid-femoral PWV

Ticagrelor and clopidogrel had no statistically significant effect adjusted for age and BP level cfPWV at baseline (9.6±1.6 versus 9.1±1.3 m/s, $P=0.19$). At 30-day follow-up, cfPWV decreased significantly in the ticagrelor group (by 0.43±0.57 m/s, $P<0.001$, by paired t test), whereas treatment with clopidogrel was associated with a mild, nonsignificant (by 0.12±0.14 m/s, $P=0.47$) increase in cfPWV.

Ticagrelor compared with clopidogrel produced a significant ($F=6.400$, $P=0.004$, by 2-way ANCOVA) decrease in cfPWV, denoting a midterm decrease in aortic stiffness (Figure 3). The interaction between treatment type and time was marginally significant ($F=3.29$, $P=0.05$). The treatment effect remained statistically significant even after adjusting for mean arterial pressure ($F=5.667$, $P=0.01$).

Inflammatory and endothelial markers

Baseline TNF-α and ADAMTS-13 (vWF) were significantly higher in the ticagrelor group compared with the clopidogrel group (all $P<0.001$), whereas endothelin-1 levels were similar between the 2 midterm study groups (Table 1). After 30 days,
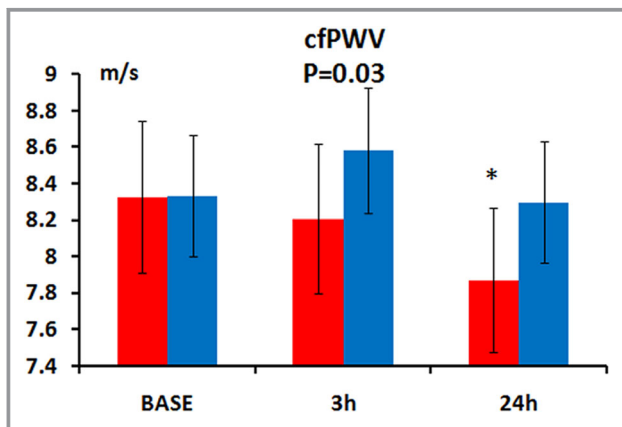

Figure 2. cfPWV according to antiplatelet loading dose (ticagrelor vs clopidogrel) measured across time points during the overall study time course. ANCOVA P value indicates the main effect (between-subjects effect) for treatment. cfPWV expressed as means; errors bars indicate SE. *$P<0.05$ in post hoc comparisons between ticagrelor and clopidogrel at 24 hours. cfPWV indicates carotid-femoral pulse wave velocity.

Table 3. Effect of Ticagrelor Versus Clopidogrel on BP and Heart Rate (Midterm Study)

	Ticagrelor		Clopidogrel		
	Base	30 Days	Base	30 Days	P Value*
Systolic BP, mm Hg	133±15	132±11	129±12	133±8	
	−0.8±14		3.5±10		0.30
Diastolic BP, mm Hg	77±10	77±9	76±9	79±8	
	−0.4±9		2.3±10		0.37
Pulse pressure, mm Hg	55±11	55±9	52±9	54±5	
	−0.3±9		1.2±9		0.64
Mean BP, mm Hg	96±11	95±9	93±9	95±12	
	−0.7±11		2.6±11		0.49
Heart rate, bpm	68±11	71±6	65±8	71±6	
	3±9		6±8†		0.28
cfPWV, m/s	9.6±1.6	9.1±1.3	9.1±1.3	9.1±1.4	
	0.43±0.57‡		0.12±0.14		<0.001

Values are mean±SD. BP indicates blood pressure; cfPWV, carotid-femoral pulse wave velocity.
*P values by 2-way ANOVA.
†$P<0.001$ and ‡$P<0.05$ compared with baseline values (paired t test).
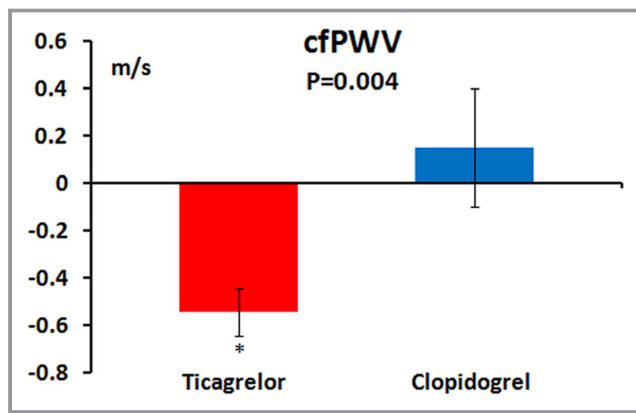
Figure 3. Comparison of changes in cfPWV at 30-day follow-up after ticagrelor and clopidogrel daily administration. *P* values at the top of graph by ANCOVA. Error bars indicate SE. *$P<0.001$ compared with baseline value (paired *t* test). cfPWV indicates carotid-femoral pulse wave velocity.

there was a nonsignificant reduction of TNF-α levels in the ticagrelor group and an increase in the clopidogrel group in comparison with baseline (-0.35 ± 0.79 versus 1.31 ± 0.90 pg/mL, $P=0.35$, by 2-way ANOVA). Changes of ADAMTS (vWF) and endothelin-1 levels 30 days after either ticagrelor or clopidogrel daily administration were minimal ($P=0.67$ and $P=0.58$, respectively; Figure S2).

Discussion

To the best of our knowledge, this is the first study showing that ticagrelor, in contrast to clopidogrel, improves aortic stiffness during the first 24 hours after a loading dose and at 1 month after continuous administration in patients undergoing coronary angiography and elective PCI. There were no significant changes between groups in both the acute and midterm study period in the pro-inflammatory and endothelial function parameters. Given the important prognostic role of aortic stiffness, these findings may have clinical implications.

Clinical Implications

Ticagrelor has a more consistent, faster-acting, and more potent antiplatelet effect than clopidogrel, which translates into improved clinical outcomes. In the PLATO trial, administration of ticagrelor showed larger benefits on hard cardiovascular end points and all-cause mortality compared with clopidogrel in ACS with or without ST-segment elevation at 12 months.[7] However, the mechanism by which ticagrelor reduces cardiovascular risk in patients is not fully understood, and whether ticagrelor may confer benefits mediated beyond its antiplatelet activity is debated.[21,29] Aortic stiffness is an independent predictor of cardiovascular morbidity and

mortality, as well as total mortality.[1–4,30] Importantly, aortic stiffness is increased in patients with CAD.[31] Elucidation of the effect of ticagrelor regarding aortic stiffness is important in order to better understand the clinical profile of this agent. The more potent acute and midterm favorable effect of ticagrelor compared with clopidogrel on aortic elastic properties imply pleiotropic effects beyond P2Y12 inhibition that may be a contributory factor in cardiovascular risk reduction reported in patients receiving this P2Y12 platelet receptor antagonist.

Mechanisms

Although the present study cannot elucidate precise mechanisms responsible for the improvement of aortic stiffness, several pathways are plausible. Physiologically, large artery stiffness depends on 3 main factors: structural elements of the arterial wall, such as elastin and collagen; distending pressure; and vascular smooth muscle tone.[5] Changes in smooth muscle tone alter the distribution of forces within the arterial wall, providing functional regulation of aortic stiffness. Our findings could be explained by adenosine-induced vasodilatory effects of ticagrelor.[14,32] Adenosine is increased locally at ischemic tissues.[33,34] Importantly, and in concordance with the above preclinical evidence, significantly higher adenosine plasma concentration has been confirmed in patients with ACS treated with ticagrelor compared with clopidogrel.[35] Interestingly, according to recent data, ticagrelor but not prasugrel increases levels of plasma adenosine in diabetic patients with ACS,[19] whereas the effect of both drugs in post-ACS individuals was not significant.[36] Nevertheless, ticagrelor has an additional mode of action, not present for the thienopyridines, because it also inhibits cellular adenosine uptake via the equilibrative nucleoside transporter 1 and thereby reduces the cellular uptake of adenosine resulting in its prolonged local $t_{1/2}$ and extracellular presence.[37]

Another potential mechanism could be the adenosine-mediated improvement of endothelial function. However, the effect of ticagrelor on endothelial function remains a controversial issue. Indeed, evidence supports a beneficial effect of ticagrelor on markers of endothelial function, such as flow-mediated dilation,[19,38] reactive hyperemia index,[22,23,35] and endothelial progenitor cells,[19,39] a finding that seems to be more prominent among high-risk subgroups of CAD patients (recent ACS, diabetes mellitus, several cardiovascular risk factors, chronic obstructive pulmonary disease). In addition, ticagrelor pretreatment improves downstream coronary vascular flow in dysfunctional vessels as compared with clopidogrel immediately after chronic coronary total occlusion recanalization.[24] On the other hand, in stabilized patients who had an ACS, ticagrelor did not improve brachial flow-mediated dilation as compared with prasugrel and clopidogrel.[36] Furthermore, in our

study, no effect was found on markers of endothelial function, such as ADAMTS-13 (vWF) and endothelin-1, in the ticagrelor group compared with the clopidogrel group in both the acute and midterm study. Accordingly, the extent of the benefit of ticagrelor on aortic elastic properties attributable to a favorable effect on endothelium is uncertain.

Inflammation causally increases cfPWV. Low-grade inflammation has been associated with both chronic arterial stiffening (via associations with inflammatory markers/mediators)[40,41] and acute arterial stiffening (via cause-and-effect in clinical experiments).[42] Reduction of systemic inflammation may partly account for the acute and midterm effect of ticagrelor on large artery stiffness. While in our study there was no significant impact on the pro-inflammatory cytokine TNF-α levels, such an effect was evident in a previous study and may be related to the specific markers studied (interleukin-6 and TNF-α).[19] Furthermore, the magnitude of decrease in pro-inflammatory cytokines may differ with regard to the patient group, being more evident in diabetic patients with non–ST-segment–elevation–ACS who are considered extremely high-risk patients with pro-inflammatory status,[19] compared with the stabilized CAD subjects who participated in our study. Undoubtedly, more likely cytokines that have been correlated in previous studies with changes in aortic wall mechanics induced by reduction of inflammation, such as interleukin-6 and interleukin-1β, should be investigated in future studies.

Strengths and Limitations

An important strength of this study is that it addresses not only the acute effect of a loading dose of ticagrelor but also the response at 1 month, a time frame that can be safely used as a basis to extrapolate on the long-term effect of the drug.

A previous study[19] reported a nonsignificant change of brachial–ankle PWV after ticagrelor as compared with prasugrel administration. The relative discrepancy regarding arterial stiffness measurements may be attributed to the different territory assessed by cfPWV and brachial–ankle PWV. Indeed, while cfPWV evaluates an elastic-type artery (aorta), brachial–ankle PWV evaluates elastic, muscular, and mixed-type arteries. Nevertheless, while brachial–ankle PWV has recently shown considerable evidence in predicting cardiovascular risk,[43] cfPWV best fits the stringent criteria of a surrogate end point.[44]

We focused on stabilized patients to minimize the risk of the natural course of the disease (ie, the acute inflammatory phase of ACS and tissue ischemia), confounding the comparison across P2Y12 inhibitors.

Limitations of our study include the following. We investigated the effect of ticagrelor versus clopidogrel on cfPWV in patients with indication for elective coronary angiography (acute study period) and with indication for either ad hoc or elective PCI (midterm study period). Whether this finding also applies in different CAD populations (for example, after an ACS) is unclear. While inferences for a long-term effect of the drug on aortic stiffness appear justified, there exists no absolute certainty.

The difference between the 2 study groups in the acute study remained nonsignificant in terms of the interaction between treatment type and time; however, this finding may be related to the relatively low number of participants. There is also a theoretical possibility of a type I error because of our relatively optimistic α-value set at α=0.05 that could lead to an overestimation of the effect. However, a detailed, a priori power analysis was conducted to ensure the reliability of our results. Furthermore, we conducted a post hoc correction of the P value to (P=0.05/2=0.025) because of the 2 time end points (3 and 24 hours) that cfPWV was examined without any significant change in the interpretation of our results.

Finally, the primary outcome of our study is the change in cfPWV. Since the sample size is modest, the secondary end points were not included in our sample size calculation and their role is explanatory. Thus, adjustment for multiple testing might overlook any possible associations that might emerge. Nevertheless, the main findings regarding secondary end points would not change, because all our results were not statistically significant.

Conclusions

The present study highlights, for the first time, a favorable effect of ticagrelor on aortic stiffness in patients with indication for elective angiography and ad hoc or elective PCI. Although no pathogenetic relationships can be established, this novel finding offers new insights into the mechanisms through which ticagrelor exerts its beneficial action. Present results warrant confirmation by long-term studies.

Sources of Funding

Disclosures

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SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

Two-Sample T-Tests using Effect Size

Numeric Results for Two-Sample T-Test
Alternative Hypothesis: H1: d ≠ 0

Target Power	Actual Power	N1	N2	N	Effect Size d	Alpha
0.80	0.8014	29	29	58	0.75	0.050

References
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Report Definitions
Target Power is the desired power. May not be achieved because of integer N1 and N2.
Actual Power is the achieved power. Because N1 and N2 are integers, this value is often (slightly) larger than the target power.
N1 and N2 are the number of items sampled from each population.
N is the total sample size, N1 + N2.
Effect Size: $d = (\mu 1 - \mu 2) / \sigma$ is the effect size. Cohen recommended Low = 0.2, Medium = 0.5, and High = 0.8.
Alpha is the probability of rejecting a true null hypothesis.

Summary Statements
Group sample sizes of 29 and 29 achieve 80.14% power to reject the null hypothesis of zero effect size when the population effect size is 0.75 and the significance level (alpha) is 0.050 using a two-sided two-sample equal-variance t-test.

Dropout-Inflated Sample Size

Dropout Rate	Sample Size N1 D2	Sample Size N2 D	N	Dropout-Inflated Enrollment Sample Size N1'	Dropout-Inflated Enrollment Sample Size N2'	Dropout-Inflated Enrollment Sample Size N'	Expected Number of Dropouts D1
3%	29 1	29 2	58	30	30	60	1

Definitions
Dropout Rate (DR) is the percentage of subjects (or items) that are expected to be lost at random during the course of the study and for whom no response data will be collected (i.e. will be treated as "missing").
N1, N2, and N are the evaluable sample sizes at which power is computed. If N1 and N2 subjects are evaluated out of the N1' and N2' subjects that are enrolled in the study, the design will achieve the stated power.
N1', N2', and N' are the number of subjects that should be enrolled in the study in order to end up with N1, N2, and N evaluable subjects, based on the assumed dropout rate. After solving for N1 and N2, N1' and N2' are calculated by inflating N1 and N2 using the formulas N1' = N1 / (1 - DR) and N2' = N2 / (1 - DR), with N1' and N2' always rounded up. (See Julious, S.A. (2010) pages 52-53, or Chow, S.C., Shao, J., and Wang, H. (2008) pages 39-40.)
D1, D2, and D are the expected number of dropouts. D1 = N1' - N1, D2 = N2' - N2, and D = D1 + D2.

Two-Sample T-Tests using Effect Size

**Procedure Input Settings**
Autosave Inactive

Design Tab
Solve For:	Sample Size
Alternative Hypothesis:	Two-Sided
Power:	0.8
Alpha:	0.05
Group Allocation:	Equal (N1 = N2)
d:	0.75

Table S1. Exclusion Criteria.

1. Who had ACS within 12 months of screening.
2. Previous stent implantation with dual antiplatelet therapy within 12 months of screening.
3. Subjects being treated with anti-platelet medications other than aspirin prior to diagnostic catheterization including glycoprotein IIb/IIIa inhibitors.
4. Subjects with NYHA class III or IV heart failure or known left ventricular ejection fraction < 30%.
5. Subjects with hemodynamic or electrical instability (including shock).
6. History of gastrointestinal bleeding, genitourinary bleeding or other site abnormal bleeding within the previous 6 months.
7. Other bleeding diathesis, or considered by Investigator to be at high risk for bleeding.
8. Any previous history of ischemic or hemorrhagic stroke, intracranial hemorrhage or disease (neoplasm, arteriovenous malformation, aneurysm).
9. International Normalized Ratio (INR) known to be >1.5 within 1 week of study entry.
10. Poorly controlled blood pressure (>160/100 mmHg).
11. Known platelet count of <100,000/mm3 within 1 week of study entry.
12. Known anemia (hemoglobin [Hb] <10 gr/dL) within 1 week of study entry.
13. Subjects receiving daily treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) or cyclooxygenase-2 (COX-2) inhibitors that cannot be discontinued for the duration of the study.
14. Severe kidney disease (GFR<30 ml/min/1.73m2).
15. Hepatic insufficiency defined as liver cirrhosis, AST/ALT/Alkaline Phosphatase greater than 3 times the upper limit of normal or hyperbilirubinemia defined as peak total serum bilirubin greater than 2 times the upper limit of normal (ULN).
16. Any indication (atrial fibrillation, mitral stenosis or prosthetic heart valve, pulmonary embolism (PE), deep vein thrombosis (DVT)) for anticoagulation treatment during study period.
17. Asthma or chronic obstructive pulmonary disease requiring brochodilating agents.
18. Concomitant use of potent Cytochrome P450 3A4 (CYP3A4) inhibitors (atazanavir, clarithromycin, indinavir, itraconazole, ketoconazole, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin and voriconazole) or inducers (carbamazepine, dexamethasone, phenobarbital, phenytoin, rifampin, and rifapentine).
19. Concomitant use of drugs that are metabolized through CYP2C19 (omeprazole and esomeprazole, fluvoxamine, fluoxetine, moclobemide, voriconazole, fluconazole, ticlopidine, ciprofloxacin, cimetidine, carbamazepine, oxcarbazepine and chloramphenicol).
20. History of uric acid nephropathy and high levels of uric acid within 1 week of study entry.
21. Increased risk of bradycardic events (e.g. known sick sinus syndrome or third degree AV block or previous documented syncope suspected to be due to bradycardia unless treated with a pacemaker).

22. Known neoplastic or autoimmune disease or any concomitant medical illness that in the opinion of the Investigator may interfere with or prevent completion in this study.

23. Contraindication to clopidogrel, ASA, or ticagrelor.

24. A history of alcohol and/or substance abuse that could interfere with conduct of the trial.

25. Pregnancy or lactation (for premenopausal women 2 methods of reliable contraception, one of which must be barrier method, are required).

26. Treatment with other investigational agents (including placebo) or devices within 30 days prior to randomization or planned use of investigational agents or devices prior to the Day 30 visit.

27. Life expectancy less than 1 year.

28. Indication for major surgery (e.g. cancer treatment, carotid surgery, cerebral surgery, major vascular surgery).

29. High likelihood of being unavailable for the Day 30 follow up.

Figure S1. Intragroup comparisons of (A) tumor necrosis factor (TNF-α), (B) a disintegrin and metalloproteinase with thrombospondin type 1 motif, 13 (ADAMTS-13) and (C) endothelin-1 across the time points in the clopidogrel (left) and ticagrelor (right) groups.
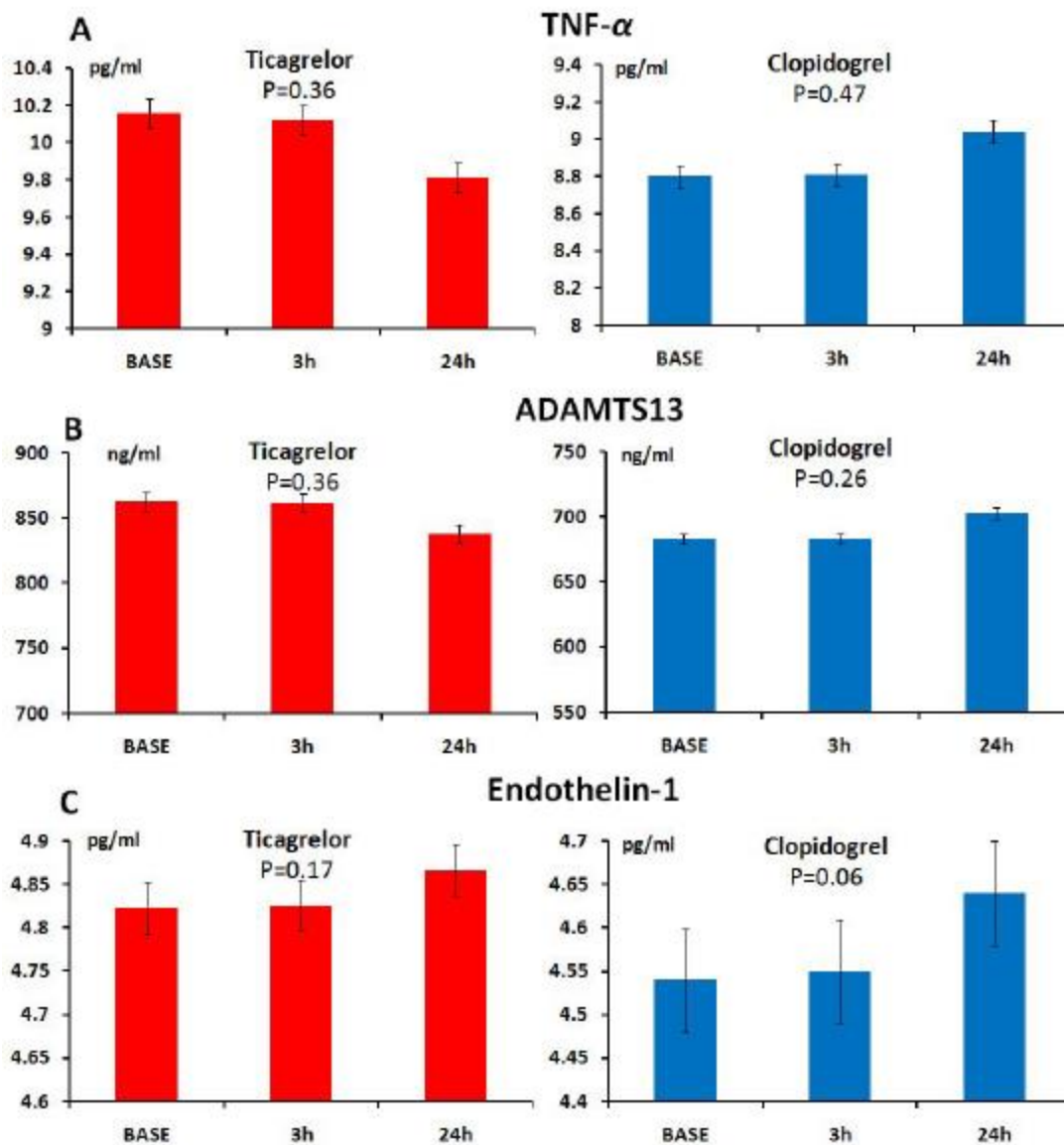
Analysis of variance (ANOVA) refers to the overall effect in TNF-α, ADAMTS-13 and endothelin-1 in each treatment group. Data are expressed as mean values; error bars indicate standard error (SE).

Figure S2. Comparison of changes in (A) tumor necrosis factor (TNF-α), (B) a disintegrin and metalloproteinase with thrombospondin type 1 motif, 13 (ADAMTS-13) and (C) endothelin-1 after 30-day ticagrelor (left) and clopidogrel (right) administration.
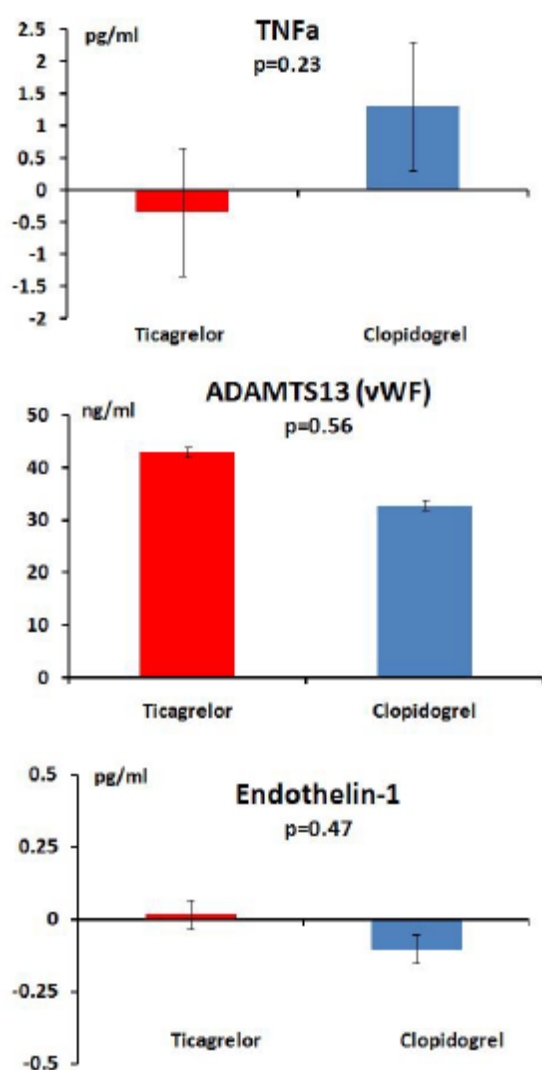
p values at the top of graphs by analysis of variance (ANOVA): error bars indicate standard error (SE).