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Effects of Ticagrelor, Prasugrel, or Clopidogrel on Endothelial Function and Other Vascular Biomarkers

A Randomized Crossover Study

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ABSTRACT

OBJECTIVES The study sought to assess whether treatment with ticagrelor, as compared with prasugrel and clopidogrel, improves endothelium-dependent dilation throughout the course of the treatment and other vascular biomarkers, including systemic adenosine plasma levels.

BACKGROUND The in vivo off-target effects of ticagrelor in post-acute coronary syndrome (ACS) patients remain poorly characterized.

METHODS Fifty-four stable post-ACS patients were sequentially exposed to each of the 3 oral $P2Y_{12}$ inhibitors following a 3-period balanced Latin square crossover design with 4 weeks per treatment in 5 European centers. The primary endpoint was the assessment of endothelial function with pulse amplitude tonometry and expressed as reactive hyperemia index at treatment steady state. Secondary endpoints included reactive hyperemia index after loading or before maintenance regimen, systemic adenosine plasma levels, a wide set of vascular biomarkers, and ticagrelor or AR-C124910XX plasma levels throughout each ticagrelor period. In 9 patients, the evaluation of endothelial function was performed simultaneously by pulse amplitude tonometry and flow-mediated dilation.

RESULTS Reactive hyperemia index did not differ after ticagrelor (1.970 ± 0.535) as compared with prasugrel (2.007 ± 0.640; $p = 0.557$) or clopidogrel (2.072 ± 0.646; $p = 0.685$), nor did systemic adenosine plasma levels or vascular biomarkers at any time points. $P2Y_{12}$ platelet reactivity units were lower after ticagrelor as compared with clopidogrel at all time points and after maintenance dose as compared with prasugrel. Flow-mediated dilatation did not differ after the maintenance dose of ticagrelor as compared with clopidogrel and prasugrel.

CONCLUSIONS Ticagrelor did not improve endothelial function or increased systemic adenosine plasma levels as compared with prasugrel and clopidogrel in stabilized patients who suffered from an ACS. (Hunting for the Off-Target Properties of Ticagrelor on Endothelial Function in Humans [HI-TECH]; NCT02587260). (J Am Coll Cardiol Intv 2018;■:■–■) © 2018 by the American College of Cardiology Foundation.

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ABBREVIATIONS AND ACRONYMS

ACS = acute coronary syndrome

ANOVA = analysis of variance

ENT1 = equilibrative nucleoside transporter 1

FMD = flow-mediated dilation

LD = loading dose

MD = maintenance dose

RHI = reactive hyperemia index

Ticagrelor, prasugrel, and clopidogrel inhibit platelet aggregation by inhibiting the adenosine diphosphate $P2Y_{12}$ receptor, and in combination with aspirin have become a class I guideline-recommended treatment in patients with acute coronary syndromes (ACS) or percutaneous coronary intervention (1).

Prasugrel and clopidogrel are thienopyridines, require conversion to an active metabolite, and mediate an irreversible inhibition of the target receptor. Ticagrelor is a nonthienopyridine direct and reversible $P2Y_{12}$ platelet receptor antagonist and, unlike prasugrel or clopidogrel, concentration-dependently inhibits the sodium-independent equilibrative nucleoside transporter 1 (ENT1) (2). This ticagrelor-mediated off-target effect has potential to increase adenosine levels, which may carry important clinical implications (2,3).

Increased adenosine levels in patients taking ticagrelor may explain some drug-specific side effects such as dyspnea and bradycardia or ventricular pauses (3). In addition, the ticagrelor-mediated increase of adenosine levels might improve endothelial function (4), a possible barometer of the total atherosclerotic risk burden (5) and this effect may contribute to explain the reduced risk of mortality observed with ticagrelor as compared with clopidogrel in the PLATO (Platelet Inhibition and Patient Outcomes) study.

There is limited and inconsistent evidence (6–8) that ticagrelor can increase adenosine plasma levels and subsequently improve endothelial function as compared with prasugrel and clopidogrel. We aimed to assess the effects of ticagrelor compared with other oral $P2Y_{12}$ inhibitors on the endothelial function, systemic adenosine plasma levels, and circulating vascular biomarkers at currently approved regimens in post-ACS patients.

METHODS

STUDY DESIGN, PROCEDURES, AND PATIENTS. The HI-TECH (Hunting for the off-target propertIes of Ticagrelor on Endothelial function and other Circulating biomarkers in Humans) trial (NCT02587260) is a randomized, open-label, crossover study conducted at 5 centers in Switzerland, the Netherlands, Spain, and Italy. Detailed inclusion and exclusion criteria were previously reported (9) and are itemized in the Online Appendix. Eligible patients were those who suffered at least 30 days earlier from an ACS, were free from ischemic or bleeding complications, and reported regular intake of dual antiplatelet therapy regimen consisting of aspirin (80 to 160 mg daily) and a clinically indicated $P2Y_{12}$ inhibitor, including ticagrelor, prasugrel or clopidogrel. After a baseline pre-randomization assessment, each patient was sequentially exposed to each of the 3 oral $P2Y_{12}$ inhibitors following a 3-period balanced Latin square crossover design with 4 weeks per treatment period. Patients were allocated in a 1:1:1:1:1:1 ratio to 1 of 6 possible treatment sequences (**Figure 1**). Allocation of study treatment was performed via an Internet-based interactive randomization system and achieved with a computer-generated random sequence with random block size, stratified according to the clinical site and the presence of diabetes mellitus.

Post-randomization measurements were performed 1 to 2 h following the loading dose (LD) of the first assigned oral $P2Y_{12}$ inhibitor (ticagrelor at 180 mg [T1] or prasugrel at 60 mg [P1] or clopidogrel at 600 mg [C1]). Patients were then requested to come back to each recruiting site 30 ± 5 days thereafter. All measurements were then repeated before (T2, P2, or C2) and 1 to 2 h after (T3, P3, or C3) the witnessed intake of the maintenance dose (MD) of the same $P2Y_{12}$ inhibitor (90 mg twice a day for ticagrelor; 10 mg/day for prasugrel, or 5 mg/day if >75 years of age or weight <60 kg; and 75 mg/day for clopidogrel). One to 7 days thereafter, patients returned to the referral hospital to receive the LD of the second randomized $P2Y_{12}$ inhibitor followed by an identical assessment algorithm until the completion of the randomized sequence (Online Appendix). No washout time was allowed before or in-between the randomized treatment sequences. Patients were requested to fast for at least 2 h before each hospital visit; caffeine-containing beverages were not permitted for 12 h before each study visit.

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FIGURE 1 **HI-TECH Trial Randomized Sequences**

30-day period / Sequence	P.1 (30±5 days)	P.2 (30±5 days)	P.3 (30±5 days)
S.1	T	P	C
S.2	T	C	P
S.3	P	T	C
S.4	P	C	T
S.5	C	T	P
S.6	C	P	T

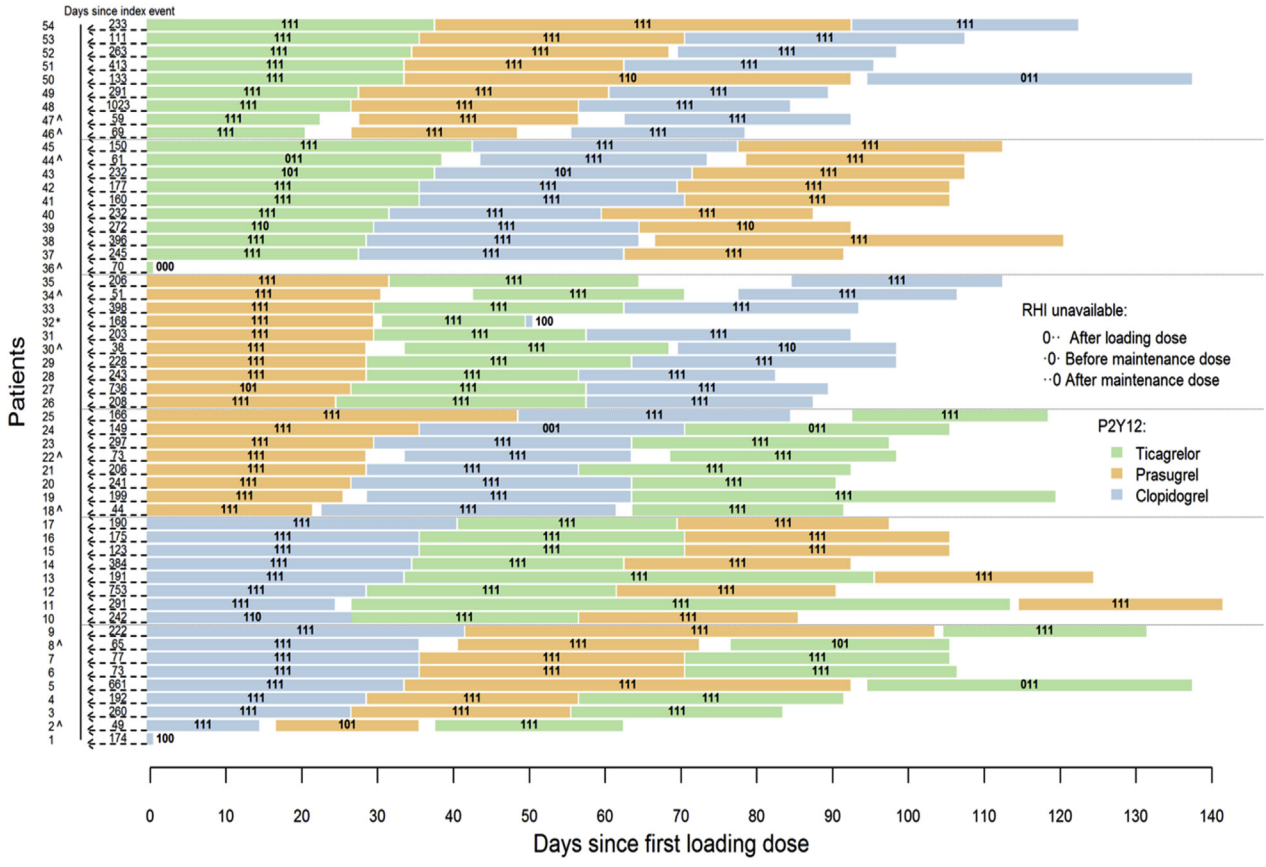
HI-TECH is a crossover study, with a Latin square design, in which every patient was randomly allocated to one of the 6 pres-pecified treatment sequences of the 3 $P2Y_{12}$ inhibitors. C = clopidogrel; HI-TECH = Hunting for the off-target propertIes of Ticagrelor on Endothelial function and other Circulating biomarkers in Humans; P = prasugrel; P.1 = first 30-day period; P.2 = second 30-day period; P.3 = third 30-day period; S.1 = first sequence; S.2 = second sequence; S.3 = third sequence; S.4 = fourth sequence; S.5 = fifth sequence; S.6 = sixth sequence; T = ticagrelor.

At baseline and each study visit, finger plethysmography assessment (EndoPAT 2000 device, Itamar Medical, Caesarea, Israel) was performed. Blood samples were taken for the assessment of vascular biomarkers (Online Appendix), systemic plasma adenosine, platelet $P2Y_{12}$ inhibitor, and aspirin functional assays (VerifyNow system, Accriva Diagnostics, San Diego, California). Ticagrelor and AR-C124910XX (ticagrelor active metabolite) plasma levels were measured at baseline and throughout each ticagrelor sequence (Online Appendix). Patients included at Bern University Hospital underwent simultaneous evaluation of endothelial function with ultrasound assessment of flow-mediated dilation (FMD) and finger plethysmography, as previously described (9) (Online Appendix).

ASSESSMENT OF ENDOTHELIAL FUNCTION. Assessment of endothelial function was obtained using pulse amplitude tonometry (primary and secondary endpoint measures), a collection of vascular biomarkers (secondary endpoint measures), and FMD of the brachial artery in a subset of patients (exploratory endpoint measures) as described (see Online Appendix). Pulse amplitude tonometry is an operator-independent, Food and Drug Administration-approved method to measure the endothelium-dependent dilation in response to reactive hyperemia (10). The pulse amplitude tonometry device records digital pulse wave amplitude using fingertip plethysmography (EndoPAT; Itamar Medical) and quantifies the endothelium-mediated changes in vascular tone, elicited by a 5-min occlusion of the brachial artery. A post-occlusion to pre-occlusion ratio is calculated by the EndoPAT software and expressed as the reactive hyperemia index (RHI). These values are normalized to measurements from the contralateral arm, which serves as a control for non-endothelial-dependent systemic effects. An RHI value of 1.670 or below denotes an abnormal endothelium-dependent dilation (endothelial dysfunction) in response to reactive hyperemia (10).

STUDY ENDPOINTS AND STATISTICAL CONSIDERATIONS. The primary endpoint was defined as RHI at treatment steady state, assessed 1 to 2 h after MD intake of the 3 $P2Y_{12}$ inhibitors (T3, P3, C3), and consisted of 2 main comparisons: ticagrelor versus prasugrel difference in RHI and ticagrelor versus clopidogrel difference in RHI. Secondary endpoints included RHI

FIGURE 2 Overview of Timing and Completeness of Study Procedures



Each **line** represents a patient. Days from index acute coronary syndrome to start of the first randomly allocated $P2Y_{12}$ inhibitor is shown on the **left-hand side** of the panel. For each $P2Y_{12}$ inhibitor sequence, duration and availability of reactive hyperemia index (RHI) assessment after the loading dose and before and after the maintenance dose is shown. For each patient, 3 (1 for each randomized $P2Y_{12}$ inhibitor) colored bars are typically shown. For each $P2Y_{12}$ sequence, the **start of the colored bar** shows when exactly the measurement was obtained after LD whereas the **end of each colored bar** shows when, before and after maintenance dose, the measurements were undertaken. After each colored bar, the **white space** shows the delay before the next randomized sequence was implemented. During these transitional periods, patients were kept on the $P2Y_{12}$ inhibitor allocated by the previous sequence (i.e., no wash-out periods). The **caret** (^) indicates patients included at Bern University Hospital who underwent flow-mediated dilation assessment, simultaneous to finger plethysmography evaluation. An **asterisk** (*) indicates that the patient did not complete C2 and C3 periods due to a skin allergic reaction to clopidogrel.

assessed 1 to 2 h after LD (T1, P1, C1), before MD (T2, P2, C2) $P2Y_{12}$ inhibitor administration, and other biomarkers of endothelial function (Online Appendix). For sample size calculation, based on a repeat 2-way analysis of variance (ANOVA) measures, we set a mean RHI of 1.800 with a within-subjects SD of 0.31 (11). With 36 patients completing all sequences (i.e., 6 patients/sequence) the study provided 90% power to detect a 10% RHI relative change in the ticagrelor group (RHI after ticagrelor MD administration) with a 2-sided alpha level at 5%. A final sample size of at least 50 patients was targeted to account for dropouts and incomplete data assessment at all time points.

The primary endpoint only of the HI-TECH trial was previously reported (12). In this paper, we report the full results of the trial including all secondary endpoints and predefined subgroup analyses.

STATISTICAL ANALYSIS. The primary endpoint was analyzed using repeated-measures 1-factorial ANOVA. The treatment factor had 3 levels: ticagrelor, prasugrel, or clopidogrel. The ANOVA yielded the differences between the 2 main comparisons: ticagrelor RHI versus prasugrel RHI and ticagrelor RHI versus clopidogrel RHI. The significance of the 2 main comparisons was combined using the Benjamini-Hochberg method to assess the primary endpoint.

TABLE 1 Baseline Characteristics, Clinical Presentation, and Baseline Medications

		Randomized Sequence					
	All Patients (N = 54)	**C-P-T (n = 9)**	**C-T-P (n = 8)**	**P-C-T (n = 8)**	**P-T-C (n = 10)**	**T-C-P (n = 10)**	**T-P-C (n = 9)**
Age, yrs	61.9 ± 10.5	67.7 ± 7.5	61.8 ± 8.0	61.5 ± 10.3	60.3 ± 14.0	61.5 ± 11.0	58.6 ± 10.5
≥75 yrs of age	7 (13.0)	3 (33.3)	0 (0)	1 (12.5)	1 (10.0)	1 (10.0)	1 (11.1)
Male	49 (90.7)	6 (66.7)	7 (87.5)	8 (100.0)	10 (100.0)	10 (100.0)	8 (88.9)
Body mass index, kg/m^2	27.9 ± 3.3	27.8 ± 2.4	25.3 ± 3.0	28.8 ± 3.8	28.2 ± 2.7	27.8 ± 2.9	29.1 ± 4.5
Diabetes mellitus	11 (20.0)	2 (22.0)	1 (13.0)	2 (25.0)	1 (10.0)	1 (10.0)	4 (44.0)
Smoker	35 (64.8)	5 (55.5)	5 (62.8)	4 (50.0)	9 (90.0)	5 (50.0)	7 (77.7)
Current	16 (30.0)	3 (33.0)	4 (50.0)	2 (25.0)	1 (10.0)	4 (40.0)	2 (22.0)
Previous	19 (35.0)	2 (22.0)	1 (13.0)	2 (25.0)	8 (80.0)	1 (10.0)	5 (56.0)
Hypercholesterolemia	26 (48.0)	5 (56.0)	1 (13.0)	5 (63.0)	6 (60.0)	4 (40.0)	5 (56.0)
Hypertension	31 (57.0)	5 (56.0)	4 (50.0)	5 (63.0)	6 (60.0)	5 (50.0)	6 (67.0)
Family history of coronary artery disease	24 (44.0)	5 (56.0)	2 (25.0)	4 (50.0)	6 (60.0)	3 (30.0)	4 (44.0)
Previous myocardial infarction	5 (9.0)	1 (11.0)	1 (13.0)	0 (0)	1 (10.0)	1 (10.0)	1 (11.0)
Previous PCI	6 (11.0)	1 (11.0)	1 (13.0)	0 (0)	2 (20.0)	1 (10.0)	1 (11.0)
Previous CABG	3 (6.0)	1 (11.0)	0 (0)	0 (0)	1 (10.0)	0 (0)	1 (11.0)
Previous TIA or stroke	2 (4.0)	1 (11.0)	0 (0)	1 (13.0)	0 (0)	0 (0)	0 (0)
Peripheral vascular disease	3 (6.0)	0 (0)	1 (13.0)	1 (13.0)	0 (0)	0 (0)	1 (11.0)
Renal failure*	3 (5.7)	0 (0)	0 (0)	0 (0)	1 (10.0)	1 (10.0)	1 (12.5)
Clinical presentation							
ST-segment elevation myocardial infarction	25 (46.0)	5 (56.0)	5 (63.0)	3 (38.0)	3 (30.0)	5 (50.0)	4 (44.0)
NSTE-ACS	23 (43.0)	3 (33.0)	2 (25.0)	4 (50.0)	5 (50.0)	5 (50.0)	4 (44.0)
Troponin positive	18 (43.0)	2 (33.0)	1 (25.0)	3 (50.0)	3 (50.0)	5 (50.0)	3 (44.0)
Troponin negative	6 (11.0)	1 (11.0)	1 (13.0)	1 (13.0)	2 (20.0)	0 (0)	1 (11.0)
Killip class >1	8 (14.8)	0 (0)	0 (0)	0 (0)	3 (30.0)	3 (30.0)	2 (22.2)
Left ventricular ejection fraction, %	51.5 ± 10.8	55.7 ± 9.9	46.4 ± 11.1	57.5 ± 7.1	47.6 ± 8.3	52.7 ± 10.8	48.7 ± 14.4
Underwent PCI	50 (96.0)	9 (100.0)	8 (100.0)	8 (100.0)	9 (90.0)	9 (100.0)	7 (88.0)
Underwent multivessel PCI	29 (56.0)	6 (67.0)	5 (63.0)	3 (38.0)	6 (60.0)	3 (33.0)	6 (75.0)
Underwent CABG	3 (6.0)	0 (0)	0 (0)	1 (13.0)	1 (10.0)	0 (0)	1 (13.0)
Time from index ACS to baseline visit							
Treatment at baseline visit, days	233 ± 189	197 ± 190	294 ± 202	172 ± 84	248 ± 199	200 ± 99	288 ± 299
Aspirin	54 (100.0)	9 (100.0)	8 (100.0)	8 (100.0)	10 (100.0)	10 (100.0)	9 (100.0)
Clopidogrel	13 (24.0)	3 (33.0)	1 (13.0)	1 (13.0)	2 (20.0)	2 (20.0)	4 (44.0)
Prasugrel	4 (7.0)	0 (0)	0 (0)	1 (13.0)	1 (10.0)	1 (10.0)	1 (11.0)
Ticagrelor	37 (69.0)	6 (67.0)	7 (88.0)	6 (75.0)	7 (70.0)	7 (70.0)	4 (44.0)
Statins	52 (96.0)	9 (100.0)	8 (100.0)	8 (100.0)	10 (100.0)	9 (90.0)	8 (89.0)
High-dose statin†	47 (87.0)	8 (89.0)	8 (100.0)	8 (100.0)	9 (90.0)	7 (70.0)	7 (78.0)
ACE inhibitor	36 (67.0)	6 (67.0)	6 (75.0)	5 (63.0)	7 (70.0)	8 (80.0)	4 (44.0)
Beta-blockers	43 (80.0)	8 (89.0)	7 (88.0)	8 (100.0)	8 (80.0)	7 (70.0)	5 (56.0)
Calcium-channel blockers	7 (13.0)	0 (0)	0 (0)	0 (0)	3 (30.0)	2 (20.0)	2 (22.0)
Angiotensin II receptor antagonists	8 (15.0)	0 (0)	0 (0)	2 (25.0)	2 (20.0)	1 (10.0)	3 (33.0)
Aldosterone antagonists	7 (13.0)	1 (11.0)	2 (25.0)	0 (0)	2 (20.0)	1 (10.0)	1 (11.0)
Proton pump inhibitors	39 (72.0)	7 (78.0)	8 (100.0)	6 (75.0)	5 (50.0)	6 (60.0)	7 (78.0)
Nitrates	5 (9.0)	0 (0)	0 (0)	1 (13.0)	1 (10.0)	1 (10.0)	2 (22.0)

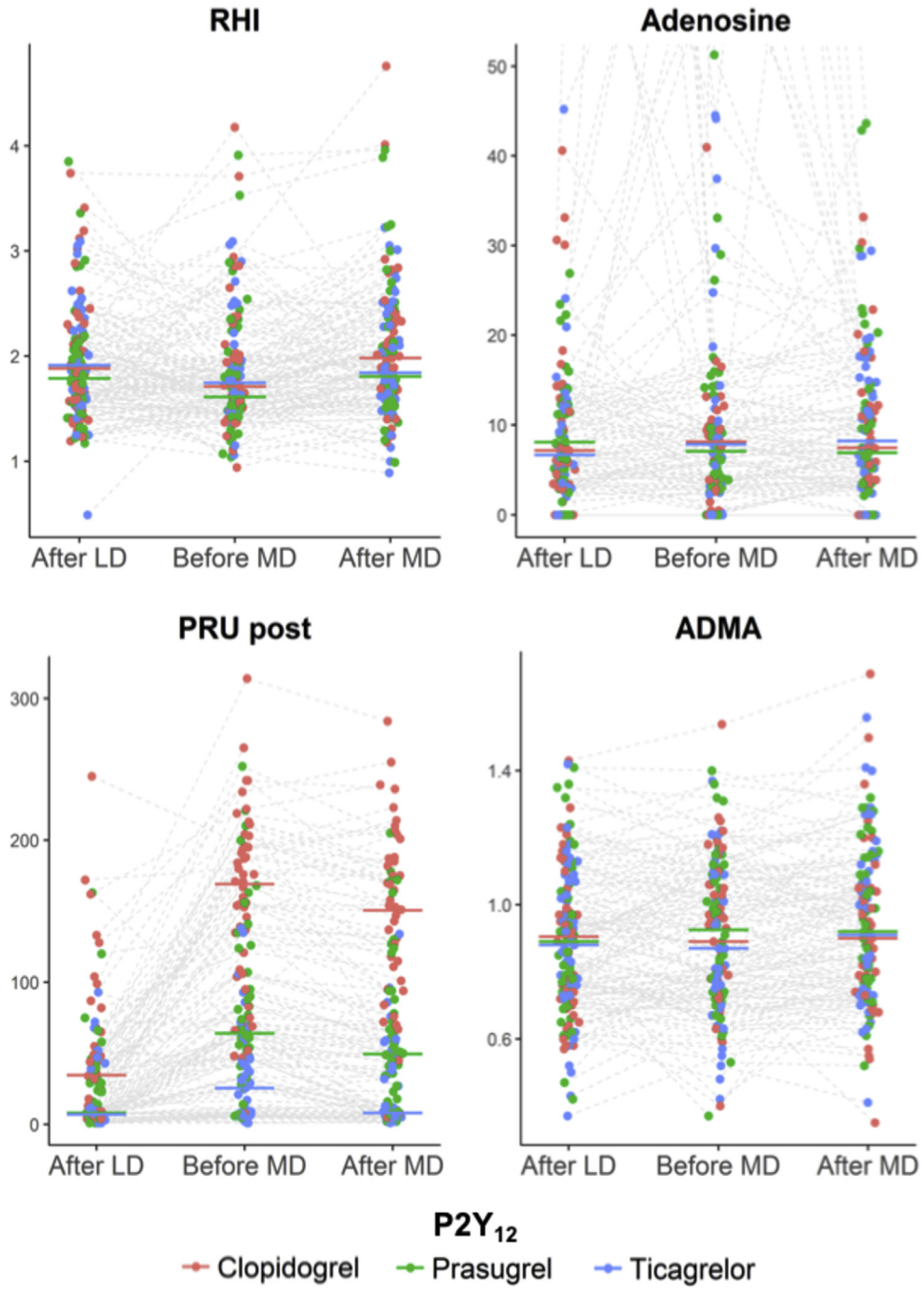
Values are mean ± SD or n (%). *Defined as creatinine clearance <60 ml/kg/min. †Defined as atorvastatin 40 mg or greater or rosuvastatin 20 mg or greater.

ACE = angiotensin-converting enzyme; ACS = acute coronary syndrome; C = clopidogrel; CABG = coronary artery bypass grafting; NSTE-ACS = non-ST-segment elevation acute coronary syndrome; P = prasugrel; PCI = percutaneous coronary intervention; T = ticagrelor; TIA = transient ischemic attack.

The null hypothesis of randomized treatment equivalence comparing the response in RHI after ticagrelor versus prasugrel and ticagrelor versus clopidogrel administration was rejected if significance was achieved for both main comparisons at a 2-sided alpha level of 0.05, or 1 comparison at a 2-sided alpha level of 0.025 (9). Within-subgroup comparisons were performed using a repeated-measures ANOVA. Additionally, p values of the interaction effect between the subgroup and the treatment factor were calculated from the repeated-measures ANOVA including the main effects and the interaction.

Correction for possible intragroup correlation was done by the Greenhouse-Geisser method. Each

FIGURE 3 **RHI, Systemic Adenosine Plasma Levels, PRU, and ADMA After Loading and Before or After Maintenance Doses of the 3 $P2Y_{12}$ Inhibitors**



Horizontal lines represent the median values at each time point for the 3 $P2Y_{12}$ inhibitors. Units for adenosine plasma and asymmetric dimethylarginine are μM. LD = loading dose; MD = maintenance dose; PRU = platelet reactivity units; RHI = reactive hyperemia index.

TABLE 2 Effect of Randomized $P2Y_{12}$ Inhibitor on Measures of Endothelial Function, Systemic Adenosine Plasma Levels, and Platelet Reactivity Units

Endpoint		Ticagrelor		Prasugrel		Clopidogrel	Global p Value	p Value Ticagrelor vs. Prasugrel	p Value Ticagrelor vs. Clopidogrel
RHI after $P2Y_{12}$ maintenance (per protocol)	48	1.840 (1.620-2.448)	47	1.810 (1.560-2.370)	47	1.980 (1.690-2.330)	0.683	0.560	0.659
RHI after $P2Y_{12}$ maintenance (matched and per protocol)	45	1.840 (1.620-2.445)	45	1.810 (1.565-2.410)	45	1.940 (1.685-2.320)	0.603	0.551	0.551
RHI after $P2Y_{12}$ maintenance if RHI <1.66	15	1.440 (1.250-1.590)	17	1.520 (1.390-1.565)	11	1.530 (1.310-1.530)	0.149	0.227	1.000
RHI before $P2Y_{12}$ maintenance	50	1.745 (1.575-2.110)	50	1.610 (1.475-1.965)	49	1.710 (1.505-2.020)	0.113	0.471	0.382
PRU post after $P2Y_{12}$ maintenance	48	8.000 (5.000-40.250)	48	49.500 (10.250-85.500)	48	150.500 (94.000-187.000)	<0.001	<0.001	<0.001
% inhibition after $P2Y_{12}$ maintenance	52	96.000 (83.250-98.000)	52	79.000 (61.250-94.500)	51	41.000 (21.000-58.000)	<0.001	<0.001	<0.001
RHI after $P2Y_{12}$ loading	49	1.910 (1.595-2.235)	52	1.785 (1.562-2.052)	51	1.880 (1.580-2.250)	0.116	0.013	0.771
PRU post before $P2Y_{12}$ maintenance	46	25.500 (7.000-50.000)	47	64.000 (33.000-107.000)	49	169.000 (107.500-195.500)	<0.001	0.006	<0.001
PRU post after $P2Y_{12}$ loading	43	7.000 (4.000-13.000)	50	8.000 (4.000-33.250)	50	34.500 (6.500-65.250)	<0.001	0.871	<0.001
% inhibition after $P2Y_{12}$ loading	50	97.000 (79.750-98.000)	51	96.000 (85.000-98.000)	53	84.000 (75.500-97.000)	<0.001	1.000	0.005
Adenosine after $P2Y_{12}$ maintenance, μM	50	8.213 (2.850-16.745)	52	6.921 (3.158-14.393)	48	7.463 (3.919-19.653)	0.163	0.878	0.200
Adenosine before $P2Y_{12}$ maintenance, μM	49	7.824 (1.295-21.746)	50	7.069 (3.130-15.597)	50	8.136 (2.747-14.023)	0.493	0.143	0.522
Adenosine after $P2Y_{12}$ loading, μM	51	6.670 (2.730-14.590)	52	8.089 (3.212-15.493)	53	7.164 (3.255-17.533)	0.703	0.644	1.000
Baseline diameter (FMD) after $P2Y_{12}$ maintenance, mm	9	3.800 (3.550-4.375)	9	3.960 (3.660-4.520)	9	3.990 (3.645-4.540)	0.051	0.070	0.180
Maximum diameter (FMD) after $P2Y_{12}$ maintenance, mm	9	3.960 (3.805-4.605)	9	4.130 (3.840-4.727)	9	4.120 (3.740-4.735)	0.555	0.453	1.000
FMD (%) after $P2Y_{12}$ maintenance	9	5.000 (4.215-6.650)	9	4.040 (3.650-5.705)	9	3.200 (1.975-4.350)	0.002	0.180	0.004

Values are n or median (interquartile range).

FMD = flow-mediated dilation; PRU = platelet reactivity units; RHI = reactive hyperemia index.

secondary endpoint was analyzed using nonparametric paired sign tests for the 2 main comparisons: ticagrelor versus prasugrel and ticagrelor versus clopidogrel. All analyses were performed on an intention-to-treat basis using Stata version 14.2 (StataCorp, College Station, Texas) and R version 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

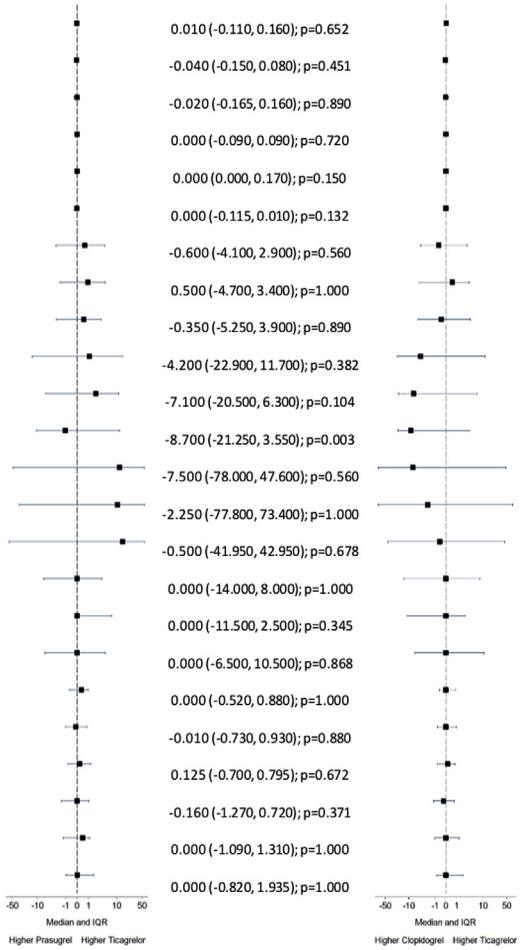
A total of 54 patients were allocated to 1 of the 6 randomization sequences from December 17, 2015, to October 25, 2016 (Online Figure 1). Of these, 50 (92.6%) patients completed the randomized $P2Y_{12}$ inhibitor sequence, and the primary endpoint measure was available for 47 (87.0%) (Figure 2).

The baseline features were similar across groups (Table 1). The mean time from index ACS to baseline visit was 233 ± 189 days, ranging from 38 to 1,023 days (Figure 2, Table 1). At the baseline visit, all patients were on dual antiplatelet therapy with aspirin (100.0%) and ticagrelor (69.0%), clopidogrel (24.0%), or prasugrel (7.0%). All patients but 2 (96.2%) were on statins and 47 (87.0%) fulfilled high-intensity criteria (Table 1). No ischemic or bleeding event was noted throughout the study.

REACTIVE HYPEREMIA INDEX. RHI after MD assessment (primary endpoint) did not differ after ticagrelor (n = 51; mean 1.970 ± 0.535) as compared with prasugrel (n = 50; mean 2.007 ± 0.64; difference: −0.048; 95% confidence interval: −0.212 to 0.115; p = 0.557) or clopidogrel (n = 49; mean 2.072 ± 0.646; difference: −0.034; 95% confidence interval: −0.200 to 0.132; p = 0.685) (Figure 3, Table 2). A matched and per-protocol analysis (sensitivity analysis) restricted to 45 within-subject comparisons across the 3 $P2Y_{12}$ inhibitors provided identical results (Table 2). The proportion of patients with on-treatment endothelial dysfunction (RHI ≤1.670) after MD assessment did not differ either across $P2Y_{12}$ inhibitors (n = 15 [29.4%] after ticagrelor; n = 17 [34%] after prasugrel; n = 11 [22.4%] after clopidogrel). RHI after LD or before MD of ticagrelor was also similar compared with prasugrel or clopidogrel (Figure 3, Table 2). Results for the

FIGURE 4 Difference in Vascular Biomarkers Measured During Each $P2Y_{12}$ Inhibitor Sequence

p-values based on paired sign tests	Ticagrelor vs Prasugrel	Ticagrelor vs Clopidogrel
Median (IQR) for the difference from Ticagrelor		
ADMA after P2Y12 maintenance (μM)	-0.025 (-0.140, 0.060); p=0.672	0.010 (-0.110, 0.160); p=0.652
ADMA before P2Y12 maintenance (μM)	-0.030 (-0.140, 0.060); p=0.371	-0.040 (-0.150, 0.080); p=0.451
ADMA after P2Y12 loading (μM)	-0.010 (-0.195, 0.115); p=0.672	-0.020 (-0.165, 0.160); p=0.890
CRP after P2Y12 maintenance (mg/dl)	0.000 (-0.150, 0.060); p=0.720	0.000 (-0.090, 0.090); p=0.720
CRP before P2Y12 maintenance (mg/dl)	0.000 (-0.090, 0.170); p=0.281	0.000 (0.000, 0.170); p=0.150
CRP after P2Y12 loading (mg/dl)	-0.020 (-0.150, 0.000); p=0.002	0.000 (-0.115, 0.010); p=0.132
Endothelin-1 after P2Y12 maintenance (pg/ml)	0.600 (-2.600, 4.300); p=0.471	-0.600 (-4.100, 2.900); p=0.560
Endothelin-1 before P2Y12 maintenance (pg/ml)	0.900 (-1.800, 4.500); p=0.291	0.500 (-4.700, 3.400); p=1.000
Endothelin-1 after P2Y12 loading (pg/ml)	0.500 (-2.550, 3.300); p=0.890	-0.350 (-5.250, 3.900); p=0.890
sICAM-1 after P2Y12 maintenance (ng/mL)	1.100 (-14.100, 14.500); p=0.888	-4.200 (-22.900, 11.700); p=0.382
sICAM-1 before P2Y12 maintenance (ng/mL)	2.100 (-5.600, 11.400); p=0.451	-7.100 (-20.500, 6.300); p=0.104
sICAM-1 after P2Y12 loading (ng/mL)	-1.050 (-10.700, 12.050); p=1.000	-8.700 (-21.250, 3.550); p=0.003
sVCAM-1 after P2Y12 maintenance (ng/mL)	11.900 (-46.500, 56.700); p=0.203	-7.500 (-78.000, 47.600); p=0.560
sVCAM-1 before P2Y12 maintenance (ng/mL)	10.500 (-32.500, 57.500); p=0.371	-2.250 (-77.800, 73.400); p=1.000
sVCAM-1 after P2Y12 loading (ng/mL)	14.650 (-58.900, 57.150); p=0.488	-0.500 (-41.950, 42.950); p=0.678
vWF after P2Y12 maintenance (%)	0.000 (-6.500, 3.500); p=0.473	0.000 (-14.000, 8.000); p=1.000
vWF before P2Y12 maintenance (%)	0.000 (0.000, 7.000); p=0.345	0.000 (-11.500, 2.500); p=0.345
vWF after P2Y12 loading (%)	0.000 (-6.000, 4.500); p=0.868	0.000 (-6.500, 10.500); p=0.868
Gpx-3 after P2Y12 maintenance (ng/mL)	0.305 (-0.590, 0.930); p=0.193	0.000 (-0.520, 0.880); p=1.000
Gpx-3 before P2Y12 maintenance (ng/mL)	-0.080 (-1.000, 0.800); p=0.761	-0.010 (-0.730, 0.930); p=0.880
Gpx-3 after P2Y12 loading (ng/mL)	0.165 (-0.730, 1.265); p=0.312	0.125 (-0.700, 0.795); p=0.672
sFLT-1 after P2Y12 maintenance (ng/mL)	-0.010 (-1.600, 1.020); p=0.771	-0.160 (-1.270, 0.720); p=0.371
sFLT-1 before P2Y12 maintenance (ng/mL)	0.410 (-1.330, 1.120); p=0.652	0.000 (-1.090, 1.310); p=1.000
sFLT-1 after P2Y12 loading (ng/mL)	0.005 (-0.970, 1.720); p=0.888	0.000 (-0.820, 1.935); p=1.000

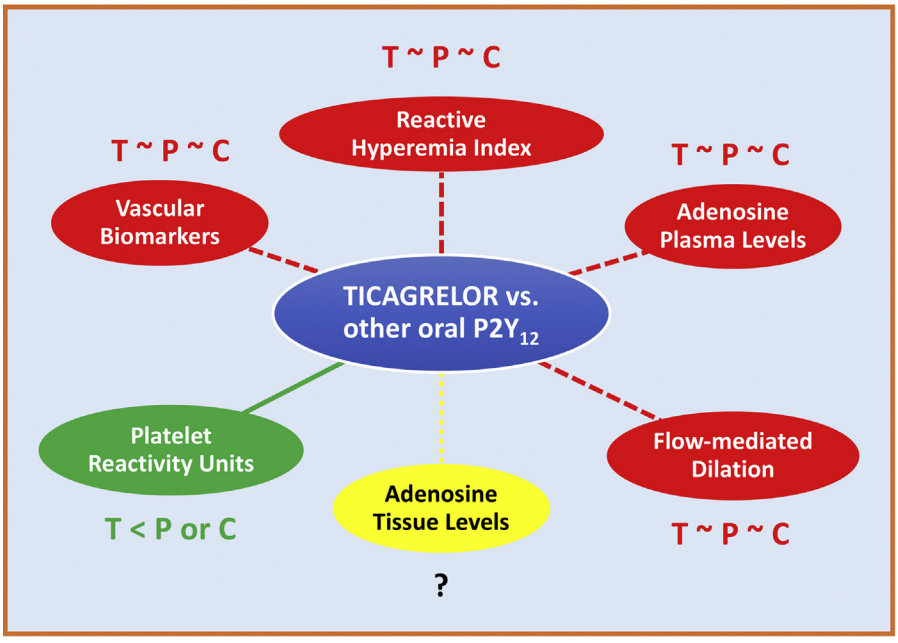


ADMA = asymmetrical dimethylarginine; CRP = C-reactive protein; Gpx-3 = glutathione peroxidase 3; IQR = interquartile range; sFLT-1 = soluble fms-like tyrosine kinase; sICAM = soluble intercellular adhesion molecule; sVCAM = soluble vascular cell adhesion molecule; vWF = von Willebrand factor antigen.

primary endpoint were consistent across multiple subgroups (Online Figure 2). There was no significant interaction between the primary endpoint and the randomized sequence ($p = 0.492$).

ADENOSINE, PLATELET REACTIVITY, VASCULAR BIOMARKERS, AND FMD ASSESSMENT. Systemic adenosine plasma levels did not differ after ticagrelor as compared with prasugrel or clopidogrel at any time point (**Figure 3, Table 2**). These results remained consistent at multiple subgroup analyses (Online Figure 3). In ticagrelor-treated patients, there was no correlation between adenosine plasma levels and ticagrelor or AR-C124910XX levels. $P2Y_{12}$ platelet reactivity units were lower after ticagrelor as compared with clopidogrel at all time points, and it was lower after MD, but not after LD as compared with prasugrel (**Figure 3, Table 2**). None of the vascular biomarkers differed after ticagrelor as compared with prasugrel or clopidogrel at any time point (**Figures 3 and 4**). In a subset of 9 patients, FMD of the brachial artery was greater after the MD of ticagrelor (median 5.00%; interquartile range: 4.20% to 6.60%) as compared with clopidogrel (median 3.20%; interquartile range: 1.98% to 4.35%; $p = 0.004$), but not as compared with

FIGURE 5 Effects of Ticagrelor Versus Other Oral $P2Y_{12}$ Inhibitors on Primary and Secondary Endpoints



The **red dashed lines and boxes** denote no difference in the effect of ticagrelor compared with prasugrel or clopidogrel. The **green line and box** indicate a higher effect of ticagrelor compared with prasugrel or clopidogrel. The **yellow dotted line and box** denote an unmeasured endpoint. C = clopidogrel; P = prasugrel; T = ticagrelor.

prasugrel (median 4.04%; interquartile range: 3.65 to 5.71; p = 0.18), nor did it differ at any other time point (**Table 2**, Online Table 1).

DISCUSSION

We conducted a randomized, open-label, balanced Latin square crossover study at 5 European centers to assess whether treatment with ticagrelor improves endothelium-dependent dilation. We also measured systemic adenosine plasma levels and several markers of endothelial function. Our findings do not support a measurable effect of ticagrelor as compared with prasugrel and clopidogrel on endothelium function, assessed by pulse amplitude tonometry, or other circulating vascular biomarkers in post-ACS patients. Systemic adenosine plasma levels did not differ at any time points. Also, none of the assessed vascular biomarkers was affected by treatment with ticagrelor as compared with prasugrel and clopidogrel. There was no signal of heterogeneity across the pre-specified subgroups for the primary endpoint or adenosine plasma levels. FMD of the brachial artery performed in a subset of patients did not provide evidence for a differential effect of ticagrelor as compared with prasugrel and clopidogrel (**Figure 5**).

Several lines of research have suggested that ticagrelor may exert an adenosine-mediated $P2Y_{12}$-independent mechanism of action. During the clinical development program of ticagrelor, dyspnea and ventricular pauses were observed in some patients and confirmed at a pivotal approval study (13). Ticagrelor was shown during in vitro experiments to inhibit adenosine uptake via inhibition of ENT1, especially if levels exceeded 1.0 μmol/l (2). In an in vitro experiment, ticagrelor added to whole blood, a high-protein binding medium, concentration-dependently conserved added adenosine. The effect was significant at ≥1.0 μmol/l, suggesting a potential effect in a clinical setting (14). In patients with ACS who received ticagrelor (90 mg twice a day) for 4 weeks, the steady-state mean of maximum plasma concentration was 1.5 μmol/l (15), suggesting that ticagrelor may exert a measurable inhibition of the ENT1 pathway in humans. However, ticagrelor and AR-C124910XX are extensively plasma protein bound in vivo (>99.7%) (2), meaning that the unbound concentration is in the low nanomolar range. Based on the

affinity of ticagrelor for the ENT1 transporter (Ki = 41 nmol/l) and the strong plasma protein binding, it was therefore anticipated that at clinically approved doses, ticagrelor could only partially inhibit ENT1 (2).

Circulating systemic plasma adenosine levels are very low in humans. However, it has been reported that they can increase locally at ischemic tissues (16). It was therefore suggested that ticagrelor can increase extracellular adenosine at tissue level through inhibition of ENT1 in erythrocytes and platelets, rather than increase systemic plasma levels (17). We did not find any measurable effect of ticagrelor on systemic adenosine plasma levels compared with the other oral $P2Y_{12}$ inhibitors, although acknowledging that adenosine tissue levels were not explored.

Ours is the third randomized trial to assess the off-target effects of ticagrelor on endothelial function, yet the first extending the comparison of ticagrelor to both prasugrel and clopidogrel, and the first multicenter trial being executed in a chronic setting, focusing on stabilized post-ACS patients. Ticagrelor was associated with an increase in adenosine plasma levels and an improvement of endothelial function at digital peripheral artery tonometry in 60 ACS patients randomized at the time of the index event to receive ticagrelor or clopidogrel (6). No crossover to the other treatment was foreseen in that study to confirm the findings. More recently, it was observed that compared with prasugrel, ticagrelor decreased inflammatory cytokines such as interleukin 6 and tumor necrosis factor alpha and increased circulating endothelial progenitor cells, contributing to improved arterial endothelial function (evaluated by FMD) in 62 diabetic ACS patients (7). In prior investigations, the duration of treatment with ticagrelor was either 4 or 5 weeks, and therefore comparable to the 4-week duration of each $P2Y_{12}$ inhibitor in our study. We recruited patients after a mean time from index ACS of 233 ± 189 days, ranging from 38 to 1,023 days. In both previous studies, patients were recruited at index admission for ACS without prior exposure to dual antiplatelet therapy. We focused on stabilized post-ACS patients to minimize the risks that the natural course of the disease (i.e., the acute inflammatory phase of ACS and tissue ischemia) may confound the comparison across $P2Y_{12}$ inhibitors and systemic adenosine plasma levels. Consistent with our results, Xanthopoulou et al. (8) observed no changes in peripheral arterial tonometry assessment before and after cessation of ticagrelor therapy in a small group of patients with stable coronary artery disease. Furthermore, our findings are also consistent with the DISPERSE-2 (Dose Confirmation Study Assessing Anti-Platelet Effects of AZD6140 vs Clopidogrel in NSTEMI 2) trial, which compared ticagrelor with clopidogrel and found no significant differences in the inflammatory biomarkers in 990 ACS patients recruited across 152 participating centers (18).

STUDY LIMITATIONS. FMD is currently the gold standard for noninvasive assessment of endothelial function in humans. We used the digital pulse amplitude tonometry as the primary endpoint of our multicenter trial because it is operator independent, reproducible, and highly correlated with FMD assessment. The pulse amplitude tonometry measures of vascular function more closely reflect basal blood flow in the brachial artery than reactive hyperemia-induced changes in the arterial diameter or flow velocity (19). The implementation of washout periods after each $P2Y_{12}$ inhibitor sequence might have allowed the assessment of carryover effects, if any, after each investigated drug. In this regard, we evaluated the interaction of the primary endpoint with the sequence, which was not significant. Patient exposure to a nonrandomly selected $P2Y_{12}$ inhibitor before the inclusion in our study has hampered the assessment of ticagrelor off-target effects when started in $P2Y_{12}$ inhibitor naïve patients during index ACS. However, despite not being randomly allocated, no effect of ticagrelor treatment versus other $P2Y_{12}$ inhibitors was noted even at baseline in our study. Our findings do not confirm previous observations that ticagrelor increases systemic adenosine plasma levels and question the existence of a measurable ticagrelor's effect on endothelial function or vascular biomarkers in stabilized post-ACS patients at currently approved regimen.

CONCLUSIONS

We found no evidence that ticagrelor exerts measurable adenosine-mediated off-target effects on endothelial function at currently approved regimen in stabilized patients who suffered from an ACS.

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PERSPECTIVES

WHAT IS KNOWN? Unlike prasugrel or clopidogrel, ticagrelor is a nonthienopyridine direct and reversible $P2Y_{12}$ platelet receptor inhibits, at least partially, the sodium-independent ENT1. This ticagrelor-mediated off-target effect has potential to increase adenosine plasma levels, which may carry important clinical implications and may explain ticagrelor-specific side effects, such as dyspnea and bradycardia or ventricular pauses.

WHAT IS NEW? In the HI-TECH trial, endothelial-dependent dilatation, assessed with the RHI, or in a subset of patients with FMD of the brachial artery, did not differ after ticagrelor as compared with prasugrel or clopidogrel in stabilized post-ACS patients at the currently approved regimen, nor did systemic adenosine plasma levels or vascular biomarkers differ at any time points.

WHAT IS NEXT? Further research is needed to assess the effect of ticagrelor on tissue adenosine plasma levels in humans compared with other oral $P2Y_{12}$ inhibitors and its relationship with clinical outcomes.

REFERENCES

1. Valgimigli M, Bueno H, Byrne RA, et al. 2017 ESC focused update on dual antiplatelet therapy in coronary artery disease developed in collaboration with EACTS: the Task Force for dual antiplatelet therapy in coronary artery disease of the European Society of Cardiology (ESC) and of the European Association for Cardio-Thoracic Surgery (EACTS). Eur Heart J 2018;39:213-60.

2. Armstrong D, Summers C, Ewart L, Nylander S, Sidaway JE, van Giezen JJ. Characterization of the adenosine pharmacology of ticagrelor reveals therapeutically relevant inhibition of equilibrative nucleoside transporter 1. J Cardiovasc Pharmacol Ther 2014;19:209-19.

3. Cattaneo M, Schulz R, Nylander S. Adenosine-mediated effects of ticagrelor: evidence and potential clinical relevance. J Am Coll Cardiol 2014;63:2503-9.

4. Torngren K, Ohman J, Salmi H, Larsson J, Erlinge D. Ticagrelor improves peripheral arterial function in patients with a previous acute coronary syndrome. Cardiology 2013;124:252-8.

5. Flammer AJ, Anderson T, Celermajer DS, et al. The assessment of endothelial function: from research into clinical practice. Circulation 2012; 126:753-67.

6. Bonello L, Laine M, Kipson N, et al. Ticagrelor increases adenosine plasma concentration in patients with an acute coronary syndrome. J Am Coll Cardiol 2014;63:872-7.

7. Jeong HS, Hong SJ, Cho SA, et al. Comparison of ticagrelor versus prasugrel for inflammation, vascular function, and circulating endothelial progenitor cells in diabetic patients with non-ST-segment elevation acute coronary syndrome requiring coronary stenting: a prospective, randomized, crossover trial. J Am Coll Cardiol Intv 2017;10:1646-58.

8. Xanthopoulou I, Vogiatzi C, Bampouri T, et al. Lack of evidence for deterioration in endothelial function following ticagrelor treatment cessation. Curr Vasc Pharmacol 2016;14:487-91.

9. Ariotti S, van Leeuwen M, Brugaletta S, et al. Rationale and design of the Hunting for the off-target propertIes of Ticagrelor on Endothelial function and other Circulating biomarkers in Humans (HI-TECH) trial. Am Heart J 2017;189: 128-36.

10. Rubinshtein R, Kuvin JT, Soffler M, et al. Assessment of endothelial function by non-invasive peripheral arterial tonometry predicts late cardiovascular adverse events. Eur Heart J 2010;31:1142-8.

11. McCrea CE, Skulas-Ray AC, Chow M, West SG. Test-retest reliability of pulse amplitude tonometry measures of vascular endothelial function: implications for clinical trial design. Vasc Med 2012;17:29-36.

12. Ariotti S, van Leeuwen M, Brugaletta S, et al. Effects of ticagrelor, prasugrel, or clopidogrel at steady state on endothelial function. J Am Coll Cardiol 2018;71:1289-91.

13. Wallentin L, Becker RC, Budaj A, et al. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. N Engl J Med 2009;361: 1045-57.

14. Nylander S, Femia EA, Scavone M, et al. Ticagrelor inhibits human platelet aggregation via adenosine in addition to P2Y12 antagonism. J Thromb Haemost 2013;11:1867-76.

15. Storey RF, Husted S, Harrington RA, et al. Inhibition of platelet aggregation by AZD6140, a reversible oral P2Y12 receptor antagonist, compared with clopidogrel in patients with acute coronary syndromes. J Am Coll Cardiol 2007;50: 1852-6.

16. Saito H, Nishimura M, Shinano H, et al. Plasma concentration of adenosine during normoxia and moderate hypoxia in humans. Am J Respir Crit Care Med 1999;159:1014-8.

17. Aungraheeta R, Conibear A, Butler M, et al. Inverse agonism at the P2Y12 receptor and ENT1 transporter blockade contribute to platelet inhibition by ticagrelor. Blood 2016;128:2717-28.

18. Husted S, Storey RF, Harrington RA, Emanuelsson H, Cannon CP. Changes in inflammatory biomarkers in patients treated with ticagrelor or clopidogrel. Clin Cardiol 2010;33: 206-12.

19. Lee CR, Bass A, Ellis K, et al. Relation between digital peripheral arterial tonometry and brachial artery ultrasound measures of vascular function in patients with coronary artery disease and in healthy volunteers. Am J Cardiol 2012; 109:651-7.

KEY WORDS adenosine, endothelial function, $P2Y_{12}$ inhibitors, prasugrel, ticagrelor

APPENDIX For expanded Methods and References sections as well as a supplemental table and figures, please see the online version of this article.