Arteriosclerosis, Thrombosis, and Vascular Biology

ORIGINAL RESEARCH

Multi-Omics Signatures Link to Ticagrelor Effects on Vascular Function in Patients With Acute Coronary Syndrome

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BACKGROUND: Long-term antiplatelet agents including the potent P2Y12 antagonist ticagrelor are indicated in patients with a previous history of acute coronary syndrome. We sought to compare the effect of ticagrelor with that of aspirin monotherapy on vascular endothelial function in patients with prior acute coronary syndrome.

METHODS: This was a prospective, single center, parallel group, investigator-blinded randomized controlled trial. We randomized 200 patients on long-term aspirin monotherapy with prior acute coronary syndrome in a 1:1 fashion to receive ticagrelor 60 mg BD (n=100) or aspirin 100 mg OD (n=100). The primary end point was change from baseline in brachial artery flow-mediated dilation at 12 weeks. Secondary end points were changes to platelet activation marker (CD41_62p) and endothelial progenitor cell (CD34/133) count measured by flow cytometry, plasma level of adenosine, IL-6 (interleukin-6) and EGF (epidermal growth factor), and multi-omics profiling at 12 weeks.

RESULTS: After 12 weeks, brachial flow-mediated dilation was significantly increased in the ticagrelor group compared with the aspirin group (ticagrelor: 3.48±3.48% versus aspirin: −1.26±2.85%, treatment effect 4.73 [95% CI, 3.85–5.62], $P<0.001$). Nevertheless ticagrelor treatment for 12 weeks had no significant effect on platelet activation markers, circulating endothelial progenitor cell count or plasma level of adenosine, IL-6, and EGF (all $P>0.05$). Multi-omics pathway assessment revealed that changes in the metabolism and biosynthesis of amino acids (cysteine and methionine metabolism; phenylalanine, tyrosine, and tryptophan biosynthesis) and phospholipids (glycerophosphoethanolamines and glycerophosphoserines) were associated with improved brachial artery flow-mediated dilation in the ticagrelor group.

CONCLUSIONS: In patients with prior acute coronary syndrome, ticagrelor 60 mg BD monotherapy significantly improved brachial flow-mediated dilation compared with aspirin monotherapy and was associated with significant changes in metabolomic and lipidomic signatures.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT03881943.

Key Words: aspirin ■ clopidogrel ■ percutaneous coronary intervention ■ phospholipids ■ ticagrelor

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Acute coronary syndrome (ACS), primarily caused by atherosclerotic plaque rupture with consequent platelet activation and aggregation and subsequent coronary thrombosis, is associated with significant mortality and morbidity.[1] Antiplatelet agents form the cornerstone of management. Contemporary guidelines recommend the use of both aspirin and a P2Y12 antagonist for 12-months after the event.[2,3] Typically, after 12-months of dual antiplatelet therapy (DAPT), patients continue aspirin monotherapy for secondary prevention. Nevertheless a significant proportion of patients with ACS develop recurrent atherothrombotic events on long-term follow-up.[4,5] Several studies have since investigated the use of prolonged DAPT including

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Nonstandard Abbreviations and Acronyms

ACS	acute coronary syndrome
DAPT	dual antiplatelet therapy
EGF	epidermal growth factor
EPC	endothelial progenitor cells
FMD	flow-mediated dilation
IL-6	interleukin-6

aspirin plus clopidogrel or ticagrelor beyond 12-months after ACS.[6,7] They demonstrated that compared with aspirin monotherapy, prolonged DAPT reduced the risk of recurrent cardiovascular events,[6,7] albeit at the expense of increased bleeding that could precipitate worse outcomes.[8] Recently, ticagrelor monotherapy has been compared with DAPT (aspirin plus ticagrelor) in patients undergoing high-risk percutaneous coronary intervention and those with ACS. Those who received ticagrelor had less clinically significant bleeding with no compromise of anti-ischemic benefit.[9–11] Moreover, a lower dose regimen (60 mg twice daily) of ticagrelor appeared to have similar therapeutic effects to a standard dose regimen (90 mg twice daily) and was associated with lower bleeding risk in patients postmyocardial infarction[12] or percutaneous coronary intervention.[13] Intuitively, the lower dose regimen (60 mg twice daily) of ticagrelor monotherapy may strike a balance between ischemia and bleeding versus aspirin monotherapy or DAPT for long-term secondary prophylaxis in post-ACS patients.

It has also been proposed that ticagrelor exhibits pleiotropic effects such as improved endothelial function beyond its potent antiplatelet effects to provide incremental clinical benefit in patients with ACS.[14,15] Nonetheless studies in patients with recent ACS have reached inconsistent conclusions about the potential pleiotropic effects including endothelial function, inflammatory parameters (eg, IL-6 [interleukin 6] and C-reactive protein) and endothelial markers (eg, endothelial progenitor cells [EPC] and EGF [epidermal growth factor]) of different P2Y12 antagonists.[16–21] This may have been due to the use of a crossover design, short study period (≈4–5 weeks) and small patient sample size.[16–20] In addition, there are no data on the effects of ticagrelor monotherapy versus aspirin monotherapy on endothelial function in patients who are stable following ACS.

In this randomized controlled study, we sought to investigate the effects of low dose ticagrelor (60 mg twice daily) monotherapy versus aspirin monotherapy on endothelial function in a group of patients with prior ACS. Endothelial function was assessed by brachial artery flow mediated dilation (FMD), platelet activation markers, plasma level of adenosine, IL-6 and circulating EPC and EGF, and multi-omics profiling.

Highlights

- Long-term antiplatelet therapy is essential in patients with history of acute coronary syndrome. This is the first study to compare the use of ticagrelor 60 mg BD monotherapy and aspirin 100 mg daily monotherapy in stable patients >18 months after prior acute coronary syndrome.
- After 12 weeks of treatment, ticagrelor monotherapy improved brachial flow mediated dilation compared with aspirin monotherapy.
- Other parameters such as platelet activation markers, endothelial progenitor cell, adenosine, interleukin-6, and epidermal growth factor appeared to have no treatment effect between 2 groups.
- Multi-omics assessment demonstrated metabolism and biosynthesis of amino acids (cysteine and methionine metabolism; phenylalanine, tyrosine and tryptophan biosynthesis) and phospholipids (glycerophosphoethanolamines and glycerophosphoserines) were associated with improved brachial flow-mediated dilation in the ticagrelor group.

METHODS

Please see the Major Resources Table in the Supplemental Material. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Design

This was a prospective, single center, parallel group, assessor-blinded, randomized controlled trial conducted at Queen Mary Hospital, Hong Kong from 2017 to 2018. Patients with a history of ACS who had completed 12-months of DAPT and had continued aspirin monotherapy for at least 6 months without further cardiovascular event were eligible for enrollment. Exclusion criteria included planned use of a P2Y12 antagonist, dipyridamole, cilostazol, vasoactive drugs, or anticoagulant therapy during the study period; bleeding disorder; history of ischemic stroke, intracranial bleeding, central nervous system tumor or intracranial vascular abnormality; or gastrointestinal bleeding within the previous 6 months or major surgery within the previous 30 days. Full inclusion and exclusion criteria are provided in the Supplemental Methods. Patients were randomized by computerized random number generation in a 1:1 ratio to ticagrelor 60 mg twice a day or aspirin 100 mg daily. Treatment allocation was open label for patients but blinded for clinical and laboratory staff who assessed outcomes. Patients were asked to return all unused study medication and empty packages to the clinic at each visit to determine their compliance with medication. All patients underwent brachial artery endothelial function assessment and fasting blood sampling at baseline and at 12-week follow-up.

The study protocol was approved by the local ethics committee, and the trial was first registered at the HKU clinical trial registry (HKUCTR-2081) in January 2017, and then also at ClinicalTrials.gov (NCT03881943) in March 2019. Informed consent was obtained from all patients.

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Study Objectives

The primary end point of the study was changes in brachial artery FMD from baseline to after 12 weeks of treatment. Secondary end points were change from baseline after 12 weeks of treatment in plasma level of adenosine and IL-6, markers of platelet activation, and circulating EPC count. We also performed exploratory untargeted multi-omics profiling analysis to evaluate pathways that potentially correlated with change in brachial artery FMD.

Brachial Artery Endothelial Function

Endothelial function was assessed by measuring brachial artery FMD as described before.[22] In brief, subjects were required to fast, to stop all medications, and to avoid smoking, drinking alcohol, and exercising for 8 hours before the measurements. All measurements were made with the patient lying in a supine position in a quiet room. Ultrasound scans were performed with a high-resolution ultrasonographic scanner (Agilent Sonos 5500; Philips, Andover, MA) equipped with a 7.5 MHz linear-array transducer. The right brachial artery was scanned over a longitudinal section 3 to 5 cm above the antecubital fossa. Both the patient's arm and the ultrasound probe were secured in position with a stereotactic clamp. A pneumatic tourniquet was held inflated around the forearm at a pressure of 50 mm Hg above the systolic blood pressure for 5 minutes. Arterial diameter was measured both at rest and after reactive hyperemia 1 minute after rapid deflation of the pneumatic tourniquet. Four measurements of arterial diameter from an end-diastolic frame (identified by the electrocardiographic R wave) were averaged to yield the brachial artery diameter during respective experimental stages. FMD was calculated as the percentage change in brachial artery diameter following reactive hyperemia compared with the baseline. Analysis of FMD was performed off-line in a random order by personnel blinded to the treatment allocation group.

Platelet Activation Assay

The degree of platelet activation was determined by flow cytometric analysis of the expression of surface expression P-selectin (CD62p) on the platelet.[23] In brief, 100 µL of whole blood was added immediately after collection to polystyrene tubes containing 50 µL HEPES buffer and 10 µL fluorochrome-labeled monoclonal antibodies. Data acquisition was performed using a flow cytometer (CytoFlex S, Beckman Coulter, Fullerton, CA). For measurement of CD62p on the platelet surface, data acquisition was triggered on forward light scatter and CD41. Platelets positive for CD62p were defined as those with fluorescence intensity above that of an isotype-matched IgG control.

EPC Count

Fluorescence-activated cell analysis was performed to determine the number of EPCs.[24] Briefly, 100 µL of peripheral blood was incubated with a phycoerythrin-conjugated monoclonal antibody against CD133 (Miltenyl), fluorescent isothiocyanate-conjugated human KDR (Sigma, St Louis, MO), and allophycocyanin-conjugated -conjugated CD34 antibody (Beckman Coulter, Fullerton, CA). PC7-labelled anti-human CD45 antibody was used for differential gating during flow analysis. APC, fluorescent isothiocyanate-labeled IgG1a (Beckman Coulter), and phycoerythrin-labeled IgG2b (Becton Dickinson, Franklin Lakes, NJ) served as the isotype controls. Analysis was performed with an automated flow cytometer (CytoFlex S, Beckman Coulter, Fullerton, CA) in which 100 000 events were counted. The absolute cell count of all the measured components per 100 000 events in the lymphocyte gate was calculated.

Plasma Level of Adenosine, IL-6, and EGF

Blood samples were obtained from subjects in the fasting state, with the first 2 mL of blood discarded and then collected in ethylenediamin tetra-acetic acid bottles. For adenosine analysis, blood was transferred into a tube with STOP solution that comprised 0.2 mmol/L dipyridamole, 4.2 mmol/L ethylenediamin tetra-acetic acid, 5 umol/L erythro-9-(2-hydroxy-3-nonyl)adenine, 79 umol/L adenosine-5'-O-(phosphonomethyl)phosphonic acid and 25 U/mL heparin (Sigma-Aldrich) in 0.9% normal saline. This induced immediate mixing of the blood with the inhibitor solution in a 2:1 blood: solution ratio. Following centrifugation, a fluorometric adenosine assay kit (Abcam) was used according to the manufacturer's instruction to detect adenosine.

Different inflammatory markers that included IL-6, C-reactive protein, and tumor necrosis factor alpha have been investigated in different studies[16,19,20] in which only IL-6 has been shown to be consistently decreased after ticagrelor treatment.[16,20] Therefore, IL-6 was measured as the inflammatory marker in this study. On the contrary, EGF is a biomarker negatively linked to endothelial function and has been shown to be decreased after ticagrelor treatment.[21] Hence, we included EGF as an additional endothelial biomarker in this study. To measure plasma IL-6 and EGF, plasma was collected following 1600*g* centrifugation at 4°C for 15 minutes. IL-6 was measured with the Cobas E diagnostic system (Elecsys IL-6, Roche Diagnostics, GmbH). Human EGF was detected using a hEGF ELISA kit (R&D systems, MN).

Multi-Omics Analysis

For metabolomics, serum was treated with 24 volumes of extract solution, mixed for 30 minutes at 4°C, and centrifuged at 12 000*g* for 10 minutes at 4°C. The supernatant was transferred into clean Eppendorf tubes. For lipidomics, serum was treated with 40 volumes of an extract solution, mixed for 30 seconds at 4°C, and centrifuged at 12 000*g* for 10 minutes at 4°C. The bottom layer of each sample was transferred into another clean Eppendorf tube. The top layer was re-extracted with chloroform. The mixture was mixed for 30 seconds and then centrifuged at 12 000*g* for 10 minutes at 4°C. The bottom layer was transferred and combined with extract from the previous tube. The supernatant was evaporated under nitrogen to dryness. The dry extracts were reconstituted with extract solution before analysis. All serum samples were combined to generate a pooled serum sample for quality control purposes and injected between every 10 sample injections to monitor the consistency of the retention time and the signal intensity. All samples were stored at −80°C before use. Following sample preparation, the sample was injected onto the filter holder of the Vanquish UHPLC system (Thermo Fisher Scientific, Germering, Germany) coupled with Thermo Q-Exactive Plus

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(Thermo Scientific, Bremen, Germany). Two runs per sample, including positive and negative ionization modes, were performed. Unlabeled peaks were extracted, peer reviewed, and representative peaks indexed manually for comparative analysis. Mobile phases for metabolomics consisted of 0.1% formic acid in water (phase A) and 0.1% formic acid in acetonitrile (phase B). The flow rate was set constantly at 0.05 mL/min, and temperature of the auto-sampler and column oven maintained at 4 °C and 25 °C, respectively, throughout the analysis. Mobile phase for lipidomics consisted of 10 mmol/L ammonium acetate in methanol. The flow rate was set and maintained at 0.1 mL/min, and temperature of the auto-sampler and column oven maintained at 4 °C and 35 °C, respectively, throughout the analysis.

Briefly, the mass spectrometer was operated in a full scan mode to continuously acquire data from the lipid chromatography eluent. The retention time-dependent data acquisition was employed using predefined retention time windows with variable widths to record the extracted ion chromatograms of untargeted analytes. Data acquisition and chromatographic peak integration were implemented using the XCalibur 4.1.50 software package from Thermo Fisher. For the untargeted global mass spectrometer analysis, >2000 features were collected and normalized as described before.[25] After multi-omics profiling, correlation and pathway analysis were performed to identify the possible underlying metabolic pathway correlating with brachial artery FMD (Figure 1).

Metabolic Pathway and Lipid Class Analyses

Metabolic pathway and lipid class analyses were carried out by annotating m/z values with a window of ±5 ppm in Kyoto Encyclopedia of Genes and Genomes database[26] for metabolites and LIPID MAPS Structure Database[27] for lipids. Default adduct lists for the corresponding polarity were generated by the xMSannotator package.[28] Then, the mapped Kyoto Encyclopedia of Genes and Genomes identifiers and LIPID MAPS Structure Database identifiers were further used to search related pathways or lipid classes, respectively. Metabolic pathways and lipid classes with 3 or more matched unique features (hits) were selected out for further analyses. Finally, feature values were aggregated into each pathway as the algorithmic mean of weighted sum by feature t-scores. All descriptive statistics and modeling of pathways and classes were performed on the aggregated data matrix.

Multi-Omics Analysis and Model Testing

With aggregated data matrix of metabolic pathways and lipid classes, a stacked Gaussian process model was fit to assess the importance scores of both pathways/classes and omics panels, as described in a previous publication.[29] Briefly, a basic Gaussian process model was first fitted with the pathways or classes from each omics panel. Next, a stacked Gaussian process model (meta-learner) was fitted with the predictions from each of the basic Gaussian models as features. Then, forward and reverse ablation analyses were performed to reveal the importance of each omics panel by recursive removal of the most or least important one from the model and seeing how the rest could make up for the loss. Finally, the features of highest importance from each basic model were displayed and studied for further discussion of biological functionalities.

Sample Size Calculation and Statistical Analysis

On the basis of our previous data,[22,24] we took a SD of 2.5% for change in FMD from baseline to 12- week follow-up. To have at least 80% power to detect a difference of 30 percentage points between the control and the treatment group with a 5% maximum false-positive error rate and using a 2-tailed test, we needed 178 patients with 1:1 randomization to aspirin treatment (n=89) and ticagrelor treatment (n=89). If a 10% dropout rate was assumed, at least 198 patients were required to be enrolled.

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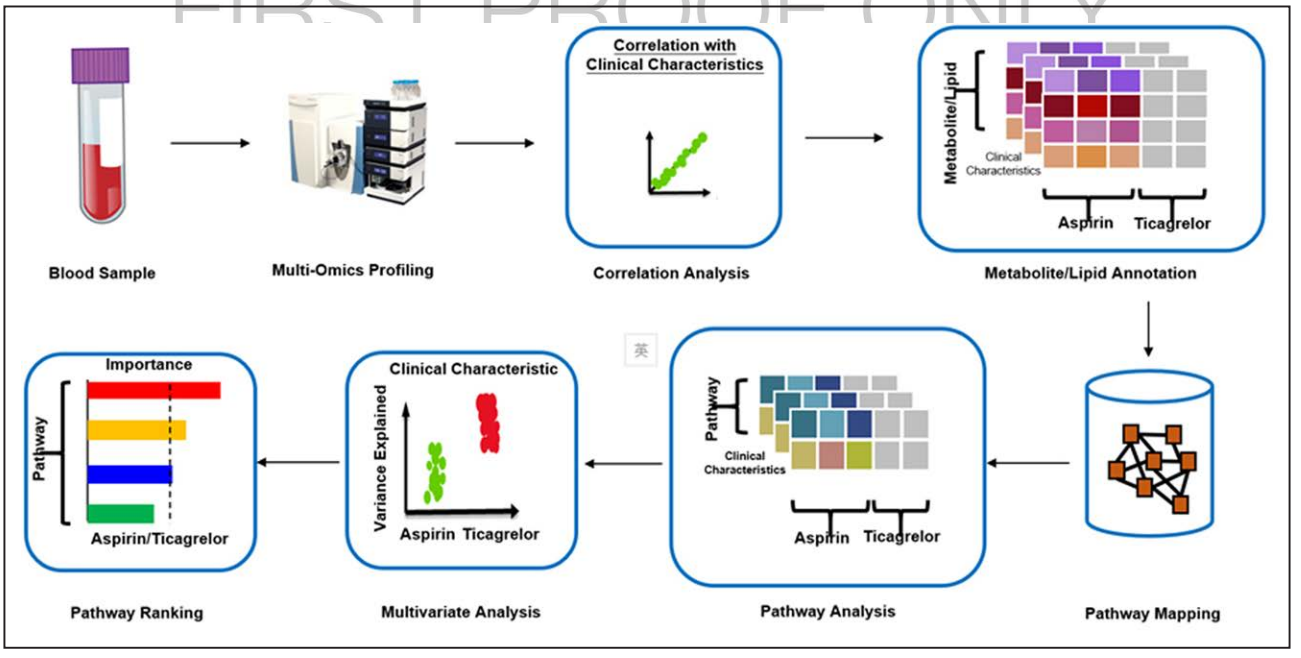

Figure 1. Workflow and design of the multi-omics analysis.

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Continuous variables with normal distribution are presented as mean and SD and variables with skewed distribution are reported as median with interquartile range. Categorical variables are described using frequencies and percentages. Comparison between groups was performed using Student *t* test for normally distributed variables and Mann-Whitney *U* test for skewed variables. χ^2 test was used to examine the association between 2 categorical variables. The analysis was performed in all randomized subjects according to the intention-to-treat principle. Paired Student *t* test (for normally distributed variables) and Wilcoxon signed-rank test (for skewed variables) was used to compare parameters before and after intervention in the two arms of the population. ANCOVA was employed to make the comparisons after adjusting for the relevant baseline values. A report of this trial fully conforms to the CONSORT guidelines.[30] Calculations were performed using SPSS software version 17.0 (SPSS Inc, Chicago, IL), and a $P<0.05$ was considered statistically significant.

RESULTS

The baseline characteristics of the study population are shown in Table 1. The mean age of subjects was 62±9 years and 87% were men. Among these patients, 25% had diabetes and 50% had hypertension. All patients were treated with aspirin at baseline and 89% were prescribed an angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, 81% a beta-blocker, and 97% a statin.

Of the 200 patients randomized in the trial, all completed final follow-up after the 12-week intervention, and 2 patients randomized to the ticagrelor group were switched back to aspirin due to drug intolerance with dyspnea (Figure 2). No patient developed any major cardiovascular or bleeding event, although 4 patients in the ticagrelor group reported mild bruises or self-resolved epistaxis.

As shown in Figure 3, brachial artery FMD at 12-week follow-up was significantly greater in the ticagrelor group compared with the aspirin group (treatment effect +4.37% [95% CI, 3.08–5.65], $P<0.001$; Table 2). Nevertheless, ticagrelor treatment for 12 weeks had no significant effect on platelet activation markers CD41_62p, circulating CD34/133 EPC count, or plasma level of adenosine, IL-6, and EGF (ANCOVA: all $P>0.05$, Table 2). The absolute treatment effect of ticagrelor on brachial FMD was inversely related to changes in plasma level of EGF (Spearman rank correlation coefficient −0.304, $P=0.003$) but not adenosine (Table S1). In addition, similar treatment effects of ticagrelor on those parameters remained unchanged after adjustment for the previous aspirin doses (Table S2A) and sex (Table S2B), as well as propensity score analyses adjusted for other drugs, age, sex, and previous doses of aspirin (Table S3A and S3B).

Table 1. Baseline Characteristics

	Ticagrelor (n=100)	Aspirin (n=100)
Mean age, y	61.2 (9.6)	62.5 (9.8)
Males, n (%)	87 (87)	86 (86)
Body weight, kg	70.4 (12.2)	70.7 (13.3)
Body height, cm	165 (17)	166 (8)
Body mass index, kg/m^2	25.4 (3.7)	25.4 (4.1)
Diabetes, n (%)	23 (23)	27 (27)
Hypertension, n (%)	46 (46)	53 (53)
Current smoker, n (%)	20 (20)	25 (25)
Past smoker, n (%)	18 (18)	24 (24)
Index coronary event, n (%)		
ST-segment–elevation myocardial infarction	70	67
Non–ST-segment–elevation myocardial infarction	21	24
Unstable angina	9	9
Mean systolic blood pressure, mm Hg	132.1 (17.6)	134.9 (18.6)
Mean diastolic blood pressure, mm Hg	82.1 (10.5)	82.1 (9.9)
Serum creatinine, mmol/dL	89 (19)	89 (20)
Mean serum low-density lipoprotein, mmol/L	1.70 (0.59)	1.79 (0.73)
Mean serum high-density lipoprotein, mmol/L	1.23 (0.38)	1.21 (0.32)
Mean serum triglyceride, mmol/L	1.40 (0.67)	1.48 (1.11)
Fasting blood glucose, mmol/L	5.67 (1.49)	5.58 (1.10)
Glycosylated hemoglobin A1c, %	6.23 (0.80)	6.17 (0.81)
Medications		
Prior antiplatelet agent, n (%)		
Aspirin	100 (100)	100 (100)
80 mg	44 (44)	40 (40)
100 mg	50 (50)	56 (56)
160 mg	6 (6)	4 (4)
Clopidogrel	54 (54)	61 (61)
Ticagrelor	40 (40)	39 (39)
Prasugrel	6 (6)	0 (0)
Angiotensin-converting enzyme-inhibitor/angiotensin receptor blocker, n (%)	89 (89)	94 (94)
Beta-blockers, n (%)	80 (80)	82 (82)
Calcium channel blockers, n (%)	14 (14)	12 (12)
Statin, n (%)	95 (95)	99 (99)
Ezetimibe, n (%)	3 (3)	4 (4)
FMD, %	2.64 (2.51)	3.65 (3.72)
CD41_62p, %	12.54 (10.94)	12.38 (10.03)
EPC count	0.35 (0.41)	0.35 (0.42)
Adenosine, μmol/L	0.67 (0.38)	0.72 (0.48)
IL-6, pg/mL	2.84 (2.36)	3.61 (3.94)
EGF, pg/mL	41.67 (54.60)	39.97 (48.01)

Data expressed as mean (SD) unless otherwise stated. EGF indicates epidermal growth factors; EPC, endothelial progenitor cells; FMD, flow-mediated dilation; and IL-6, interleukin 6.

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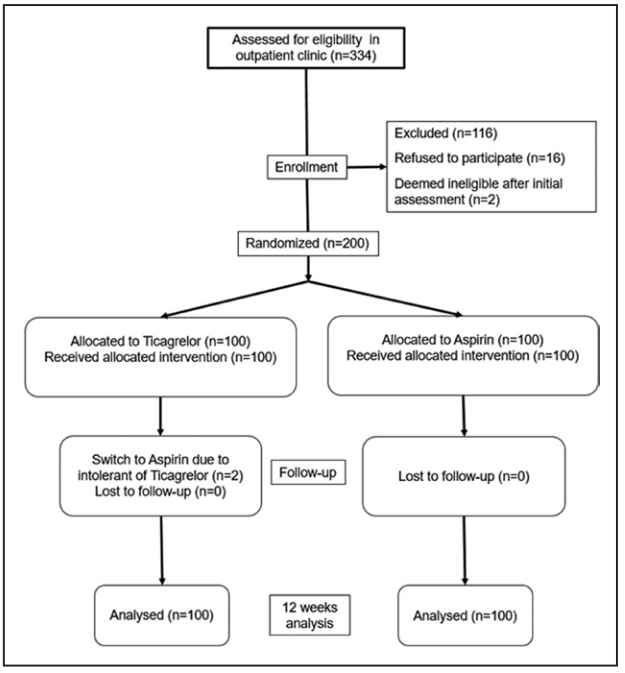

Figure 2. Flow chart of the study.

As shown in Figure 4A, initial multi-omics analysis identified a significant contribution of metabolomics and lipidomics to variance of brachial artery FMD only in the ticagrelor group. Further pathway analysis revealed that changes in the metabolism and biosynthesis of amino acids (cysteine and methionine metabolism; phenylalanine, tyrosine, and tryptophan biosynthesis) and phospholipids (glycerophosphoethanolamines and glycerophosphoserines) were associated with the improved brachial artery FMD in the ticagrelor group (Figure 4B).

DISCUSSION

To the best of our knowledge, this is the first study to compare the effects of a low dose regimen of monotherapy with ticagrelor versus aspirin on vascular endothelial function in patients with prior ACS. In this randomized controlled study, we have demonstrated that ticagrelor significantly improved vascular endothelial function compared with aspirin. Although there were no significant changes to platelet activation markers, circulating EPC or plasma level of adenosine, IL-6 and EGF, multi-omics profiling revealed that improved brachial artery FMD with ticagrelor was associated with significant changes in metabolomic and lipidomic signatures. These findings provide novel mechanistic insight into the potential beneficial effects of ticagrelor on vascular endothelial function beyond its antiplatelet action.

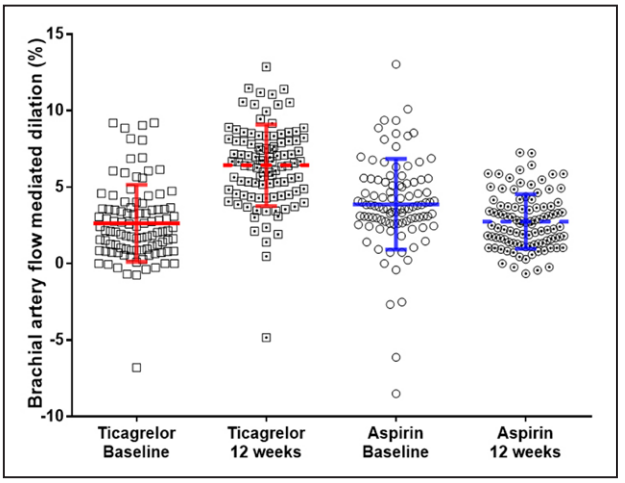

Figure 3. Brachial flow-mediated dilation at baseline and after 3 months in the ticagrelor group and the aspirin group.

P2Y12 monotherapy, in particular ticagrelor, has emerged as an attractive option, with a potent direct P2Y12 receptor antagonist effect and consistent platelet inhibition. The low dose ticagrelor monotherapy may strike an optimum balance between ischemia and bleeding as long-term treatment for post-ACS patients. Prior studies have proposed that the pleiotropic effects of ticagrelor on vascular endothelial function via adenosine metabolism can contribute to additional clinical benefits in post-ACS patients.[14,15,31] Nonetheless subsequent studies in patients with recent ACS of the impact of DAPT with different P2Y12 antagonists on vascular endothelial function, plasma adenosine, inflammatory biomarkers, and EPCs have yielded inconsistent findings.[16–20] Of note though, none of these studies compared the effect of a P2Y12 antagonist versus aspirin monotherapy on vascular endothelial function in patients with prior ACS after completion of DAPT.

In this study, all patients were stable on optimal medical therapy after a prior ACS (minimal 18 months). Furthermore, a lower dose regimen of ticagrelor 60 mg twice daily was prescribed. This is the current recommended dose for extended treatment beyond the initial 12 months after the index ACS event.[3] Our results demonstrate that ticagrelor 60 mg twice daily monotherapy significantly improved brachial artery FMD after 3 months of treatment compared with aspirin monotherapy. Nonetheless, we observed no treatment effect on different targeting biomarkers, including platelet activation markers, circulating EPC and plasma levels of adenosine, IL-6, and EGF that have been proposed to contribute to the pleiotropic effects of ticagrelor.[15–20,31,32] FMD is a surrogate marker of vascular endothelial function and regulated by numerous factors including vascular tone, cell growth, and platelet and leukocyte interactions. In addition, FMD can be mediated by endothelial-dependent and endothelial-independent pathways that involve a complex interplay of various elements.[33] Although prior studies suggested that the improvement in vascular function by P2Y12 inhibitors might be linked to their antiplatelet effect,[20] the absence of significant treatment effect on platelet activation markers in this study may support the improvement of endothelial function by ticagrelor via direct endothelial-dependent and endothelial

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Table 2. Twelve-Week Treatment Effects of Ticagrelor Versus Aspirin on Brachial Endothelial Function, and Other Parameters

	Change		Treatment effects*	
	Ticagrelor (n=100)	Aspirin (n=100)		*P* value
FMD (%)	3.79 (3.63)	−0.58 (5.40)	4.37 (3.08 to 5.65)	<0.001
CD41_62p, %	−3.41 (10.06)	−2.37 (10.06)	−1.05 (−3.91 to 1.81)	0.47
EPC count	−0.06 (0.49)	−0.08 (0.43)	0.04 (−0.08 to 0.17)	0.50
Adenosine	0.11 (0.40)	0.13 (0.49)	−0.03 (−0.15 to 0.10)	0.69
IL-6	0.16 (2.24)	−0.44 (2.98)	0.60 (−0.19 to 1.40)	0.14
EGF	−8.68 (59.15)	−8.10 (48.53)	−0.58 (−15.67 to 14.50)	0.94

Data expressed as mean (SD) unless otherwise stated. EGF indicates epidermal growth factors; EPC, endothelial progenitor cells; FMD, flow-mediated dilation; and IL-6, interleukin 6.

*Treatment effects expressed as differences in least-squares means (ANCOVA model) with 95% CI.

independent mechanisms. In this study, there was a modest but significant correlation of increased brachial FMD with decreased plasma level of EGF after treatment with ticagrelor. This finding suggests that lowering circulating EGF level might contribute partly to the improvement by ticagrelor in brachial FMD. Previous studies in patients with stable coronary artery disease and chronic inflammation due to chronic obstructive pulmonary disease showed that treatment with ticagrelor improved surrogate markers of endothelial function, mediated by induction of the SIRTI/HESI axis in reducing EGF, independent of an antiplatelet effect.[21,34] It is possible that ticagrelor exhibits a more prominent effect on EGF level in those patients with underlying chronic inflammation, and thus no significant treatment effect was observed in this study. Similarly, the lack of treatment effects of ticagrelor on platelet activation, circulating EPC, and IL-6 as reported in other studies[15,16] may be attributed to the low baseline levels of platelet activation and inflammation in our study population. In this study, all patients were on stable doses of aspirin for at least 18 months after ACS with no recurrence of ischemic events before study enrollment. Indeed, we observed a low baseline plasma level of IL-6 as well as expression of platelet activation markers CD41_62p in our patients, and thus any potential effects on those parameters by ticagrelor treatment are difficult to determine. Although our further analysis after adjustment for the previous dose of aspirin used also failed to demonstrate any significant treatment effects of ticagrelor on those parameters, we cannot exclude the confounding effect of previous chronic aspirin therapy as it was impractical to have a washout period of antiplatelet agents before randomization in our patients with prior ACS. In addition, it remains unclear whether the lower dose of ticagrelor used in this study compared with previous studies[15,16] might have also contributed to the lack of change in those parameters. These observations led to our hypothesis that other novel mechanisms may contribute to the beneficial effect of ticagrelor on vascular endothelial function.

In this study, using paired serum samples before and after ticagrelor treatment with aspirin as control, we performed unbiased metabolomics and lipidomics analysis to identify potential pathways that might account for the variance in vascular endothelial function after ticagrelor treatment. Our analysis revealed 2 major changes to the metabolomics and 2 to lipidomics signatures that were associated with the improved brachial artery FMD in the ticagrelor group. First, we determined that changes to the cysteine and methionine metabolic pathway correlated with the difference in FMD after ticagrelor. Homocysteine is an important product of the cysteine and methionine metabolic pathway. Elevation of homocysteine level impairs vascular endothelial function by decreasing the bioavailability of nitric oxide and inducing oxidative stress, endothelial apoptosis, and chronic inflammation.[35,36] Homocysteine is formed by the demethylation of methionine, via formation of S-adenosylhomocysteine, and further converted into homocysteine and adenosine by S-adenosylhomocysteine hydrolase.[37] Prior studies have shown that ticagrelor affects the cellular transport of adenosine via inhibition of equilibrative nucleoside transporter 1.[31] Accumulation of intracellular adenosine due to decreased cellular release of adenosine via equilibrative nucleoside transporter 1 inhibition by ticagrelor can affect the metabolism of homocysteine, and thus contribute to the improved endothelial function.[37] Second, phenylalanine, tyrosine, and tryptophan biosynthesis were significantly affected by ticagrelor. It has emerged that metabolism of tryptophan can play an important role in vascular homeostasis via modulation of inflammation.[36] Recent studies showed that expression of indoleamine 2,3-dioxygenase 1, which is a rate-limiting enzyme of the kynurenine pathway of tryptophan metabolism in the macrophages within atherosclerotic plaques, was closely associated with its tissue factor expression.[38] Interestingly, preclinical and human studies have shown that ticagrelor, but not clopidogrel, reduces the expression of endothelial tissue factor.[39,40] Thus, the suppression of tissue factor may have altered the expression of activation indoleamine 2,3-dioxygenase 1, and thus the metabolism of tryptophan. Third, for lipidomics, changes in glycerophospholipid metabolism were associated with

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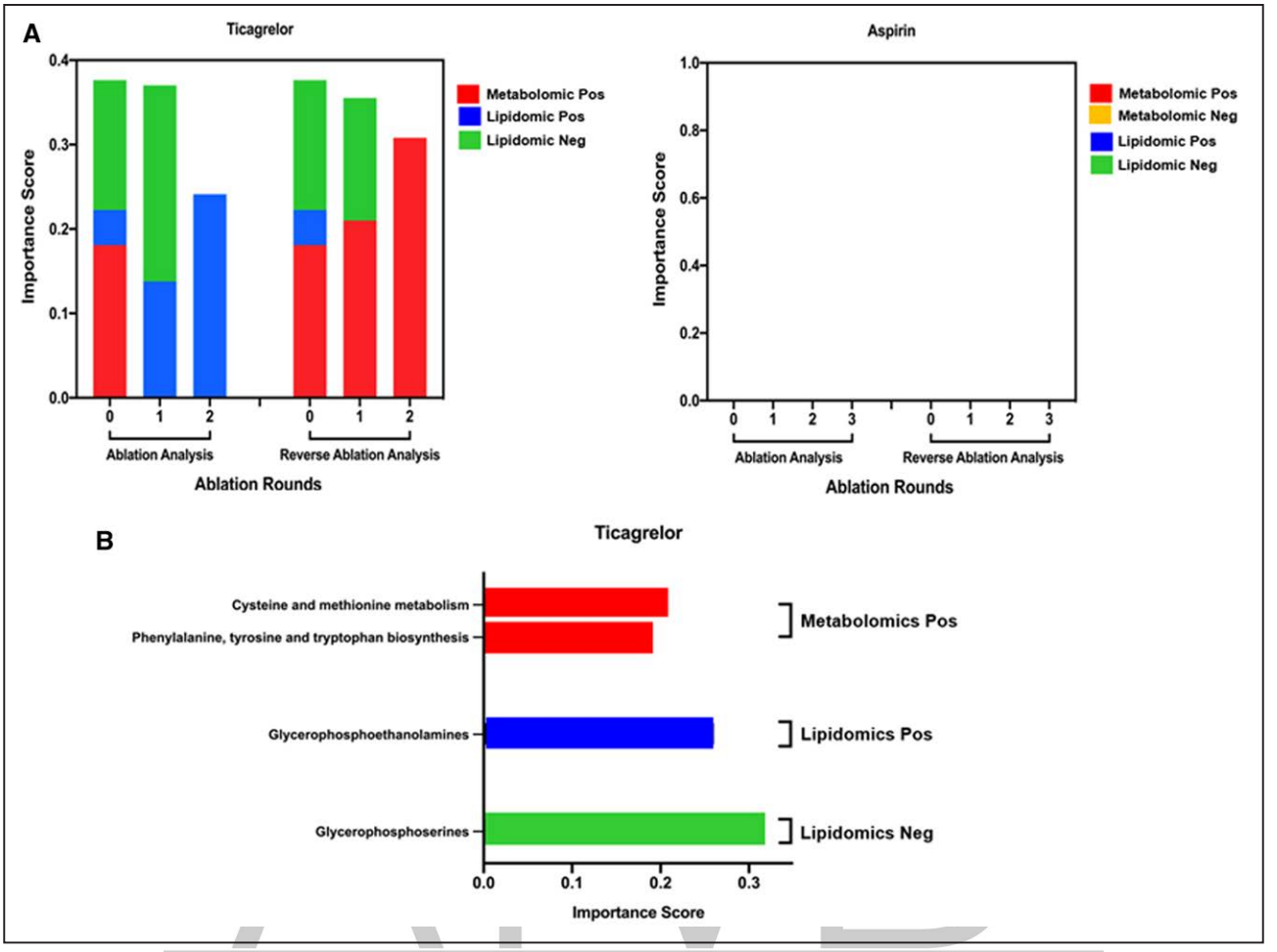

Figure 4. A, Multi-omics analysis shows the contribution of metabolomics and lipidomics to the variance in brachial artery flow-mediated dilation (FMD) in the ticagrelor group and aspirin group.
Although changes to metabolomic and lipidomic profiles were observed in the ticagrelor group, there were none in the aspirin group. **B**, Further pathway analysis demonstrates the changes in the metabolism and biosynthesis of amino acid and phospholipid associated with the improved brachial artery FMD in the ticagrelor group.

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brachial endothelial function after ticagrelor. Although the function of glycerophosphoserines remains unclear, glycerophosphoethanolamine belongs to a class of plasmalogen that is abundantly present in the membrane structure and has been linked to oxidation of low-density lipoprotein in atherosclerotic lesions in humans.[41] The mechanism by which ticagrelor alters glycerophospholipid metabolism remains unclear, but it might contribute to the bactericidal activity with inhibition of biofilm growth by ticagrelor.[42] Furthermore, glycerophosphoserine is also known as phosphatidylserine that is abundant in the membrane of the extracellular vesicles derived from activated platelets.[43] Recent studies have demonstrated that ticagrelor attenuates the release of procoagulant and proinflammatory P-selectin/phosphatidylserine positive extracellular vesicles from activated platelets in normal subjects[43] and patients with ACS.[44] This might also have contributed to the improved vascular endothelial function observed in this study. All these data are plausible biological explanations, and further studies are needed to reveal the underlying biochemical pathways of the pleiotropic effects of ticagrelor.

Our study has several limitations. First, as the sample size calculation was based on the change in brachial FMD, this study may have been underpowered to detect changes to other serum biomarkers. Second, to date, long-term ticagrelor 60 mg BD monotherapy in patients with prior ACS has not been fully investigated and represented in clinical trials. The long-term safety and efficacy of ticagrelor 60 mg BD in patients with prior ACS needs to be further investigated in future trials before clinical translation of our findings. Third, our study included a selected low-risk population with ACS in whom none had any recurrent ischemic events before enrollment and 18 (9%) had unstable angina with no previous myocardial injury. Our findings may not be applicable to high-risk ACS patients with recurrent ischemic events. Fourth, we did not determine whether genetic polymorphism of ticagrelor metabolism could affect the outcome parameters, especially when a lower dose of

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ticagrelor 60 mg BD was used in this study. Fifth, we did not elicit changes in FMD with nitroglycerine[45] to study a possible endothelium-independent effect nor attempt to measure microvascular endothelial function[46] parameters. These may have helped further elucidate whether the underlying mechanisms of FMD improvement with ticagrelor monotherapy were mediated by endothelial-dependent or endothelial-independent pathways. Finally, untargeted multi-omics analysis was performed. Targeted metabolomics and lipidomics analysis in a larger patient population is needed to provide more detailed insight into the effects of ticagrelor.

In conclusion, in patients with prior ACS, ticagrelor 60 mg BD monotherapy significantly improved brachial endothelial function compared with aspirin monotherapy. The beneficial effect of ticagrelor on endothelial function was not related to altered platelet activation, circulating level of EPC or plasma level of adenosine, but was associated with significant changes in metabolomic and lipidomic signatures. Whether these pleotropic effects of ticagrelor monotherapy can translate into improved long-term clinical outcomes in patients with prior ACS needs to be addressed by future clinical trials.

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Supplemental Materials

Supplemental Methods
Tables S1–S3
Major Resources Table

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