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Trial Design

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

The HI-TECH Investigators

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ABSTRACT

Background: Amongst the 3 approved oral $P2Y_{12}$ inhibitors for the treatment of patients with acute coronary syndrome (ACS), ticagrelor, but not prasugrel or clopidogrel, has been associated to off-target properties, such as improved endothelial-dependent vasomotion and increased adenosine plasma levels.

Methods: The Hunting for the off-target propertIes of Ticagrelor on Endothelial function and other Circulating biomarkers in Humans (HI-TECH) study (NCT02587260) is a multinational, randomized, open-label, cross-over study with a Latin squares design, conducted at 5 European sites, in which patients free from recurrent ischemic or bleeding events ≥30 days after a qualifying ACS were allocated to sequentially receive a 30±5-day treatment with prasugrel, clopidogrel, and ticagrelor in random order. The primary objective was to evaluate whether ticagrelor, at treatment steady state (i.e. after 30±5 days of drug administration), as compared with both clopidogrel and prasugrel, is associated with an improved endothelial function, assessed with peripheral arterial tonometry., Thirty-six patients undergoing evaluable endothelial function assessment for each of the assigned $P2Y_{12}$ inhibitor were needed to provide 90% power to detect a 10% relative change of the reactive hyperemia index (RHI) in the ticagrelor group.

Conclusion: The HI-TECH study is the first randomized, cross over study aiming to ascertain whether ticagrelor, when administered at approved regimen in post-ACS patients, improves endothelial function as compared with both clopidogrel and prasugrel.

Word count abstract: 218 words

Key words: endothelial function, acute coronary syndromes, off-target properties, ticagrelor, reactive hyperemia index, adenosine, pulse amplitude tonometry, flow mediated dilation.

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Highlights

- The off-target properties of ticagrelor on endothelial function are poorly studied
- HI-TECH compares ticagrelor, prasugrel and clopidogrel in post-ACS patients
- The principal endpoint of the study is the effect on endothelial function evaluated with pulse amplitude tonometry
- Measurement of adenosine plasma levels is a key secondary endpoint
- Platelet reactivity will be also assessed

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Background

Oral $P2Y_{12}$ inhibitors are key secondary prevention medications after coronary stent implantation or acute coronary syndromes (ACS). Ticagrelor, unlike other oral $P2Y_{12}$ inhibitors, has been associated with off-target effects, such as an improvement in endothelial function assessed by peripheral arterial tonometry, as recently reported in a non-randomized trial (1). Moreover, in a recent randomized trial of 60 ACS patients, 30-day ticagrelor administration was shown to increase reactive hyperemia index (RHI) by 100% as compared to baseline measurement, and this improvement to be correlated to adenosine plasma levels (2). A recent observational study also reported no change in endothelial function at 2 and 5 days after treatment discontinuation with ticagrelor, which was interpreted as evidence that the treatment effect on endothelial function does not immediately cease after treatment cessation (3).

Endothelial dysfunction is a systemic condition mainly characterized by an imbalance between endothelium-derived relaxing factors (i.e. nitric oxide) (4) and endothelium-derived contracting factors (i.e. endothelin) (5), clinically correlated with most cardiovascular risk factors(6). Endothelial dysfunction appears to precede the clinical manifestation of atherosclerotic disorders and predicts clinical outcome (7). Thus, it can be considered a barometer of the total risk burden (8-10).

The “ticagrelor-related pleiotropic effects” are possibly mediated by the inhibition of adenosine uptake into erythrocytes and subsequent increase in adenosine plasma levels (APL), as recently reported (2,11,12).

Adenosine is released in the plasma by endothelial cells and myocytes during ischemia, hypoxia, or oxidative stress, and quickly taken up by red blood cells through a facilitated diffusion transport system (sodium-independent equilibrative nucleoside transporters – 1 (ENT-1) and 2 (ENT-2); sodium-dependent concentrative nucleoside transporters – 1 and 2) or converted into inosine by adenosine deaminase activity. Ticagrelor increases APL mainly

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through inhibition of ENT-1 (11). After binding to 4 different purinergic receptors (A_1, A_{2A}, A_{2B}, A_3), an increase in APL may determine: 1) vasodilation; 2) reduction in ischemia/reperfusion injury and electrical conduction; 3) increase of platelet inhibition; 4) decrease of glomerular filtration rate; 5) rise of dyspnoea incidence. Finally, ticagrelor induces adenosine tri-phosphate (ATP) release from human red blood cells in a dose-dependent manner (13), which may contribute to increasing APL. It remains however unclear whether the off-target properties of ticagrelor, which were discovered and characterized in animals or ex vivo models (14,15), contribute to its clinical effects in humans at currently approved regimen.

Methods

Study design and population

The HI-TECH study (ClinicalTrial.gov NCT02587260) is a multinational, randomized, open-label, crossover study with a Latin squares design conducted at 5 European sites and including 54 patients, aiming to assess whether ticagrelor, as compared to both clopidogrel and prasugrel, improves endothelial function. Eligible patients were older than 18 years, treated for an ACS, including ST segment elevation myocardial infarction (STEMI), non-ST segment elevation myocardial infarction (NSTEMI), or unstable angina (UA), at least 30 days before randomization, and receiving dual antiplatelet therapy (DAPT) since at least 30 days prior to randomization. Patients were free from bleeding events [defined as Bleeding Academic Research Consortium (BARC) classification (16) type 2 or greater] or ischemic recurrences (unstable angina or myocardial infarction, which required a repeated revascularization) since the index event. Patients with transient ischemic attack (TIA) or stroke in the previous 6 months, those who received fibrinolytic therapy or glycoprotein IIb/IIIa inhibitors in the previous 30 days, those with indication for oral anticoagulant therapy, or with vasculitis, immunological disorders, thrombocytopenia, severe hepatic failure, uncontrolled hypertension

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(systolic or diastolic pressure > 180 mmHg or 120 mmHg, respectively, despite medical therapy), known intolerance to aspirin, clopidogrel, prasugrel, or ticagrelor, limited life expectancy (e.g. neoplasm) as well as patients who underwent major surgery within 30 days before randomization or with any planned surgical or percutaneous intervention were excluded (**Tab. 1**).

A Latin square design was used in order to have a uniform crossover design in that each treatment occurred only once within each sequence and once within each period. Moreover, each treatment preceded every other treatment the same number of times (twice) and, consequently, our design was balanced with respect to first-order carryover effects (balanced Latin square design). The crossover study design was chosen so to have an "in within" control of the effect of each $P2Y_{12}$ inhibitor on endothelial function (in the same patient) as opposed to an "in between" control (different patients). **Supplementary Table 1** displays the 6 randomized treatment sequences. Adherence to study treatment was assessed by electronic Medication Event Monitoring System (MEMS), pill count, and patient interview (see Suppl. Appendix).

Follow-up visits

The study included 6 follow-up visits counting a total of 10 study intervals: baseline (B), 1-2 hour(s) after LD intake of each $P2Y_{12}$ inhibitor (C1, P1, T1), before (C2, P2, T2) and 1-2 hour(s) after MD intake of each $P2Y_{12}$ inhibitor (C3, P3, T3) (**Fig. 1**). The LD of the first randomized $P2Y_{12}$ inhibitor was to be administered at visit 1 (V1), after baseline assessment, followed by post-LD measurements 1-2 hour(s) thereafter (**Fig. 1**). Pre- and post-MD evaluations of the ongoing $P2Y_{12}$ inhibitor were to be performed after 30±5 days of treatment at visit 2 (V2). The administration of the second randomized $P2Y_{12}$ inhibitor at visit 3 (V3) was to occur 1 to 7 days after V2, and the related measurements were collected 1-2 hour(s) after LD administration (**Fig. 1**). An identical scheme was then to be followed for the second

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and third randomized $P2Y_{12}$ inhibitor (**Fig. 1**). No washout time was allowed among the three oral $P2Y_{12}$ inhibitors due to ethics considerations. At each follow-up, patients were requested to fast for at least 2 hours prior the visit; caffeine-containing beverages were not permitted at any time the day of the visit. Patients were reminded 1-2 days prior each study visit about these dietary restrictions.

Finger Plethysmography

Pulse amplitude tonometry (PAT) is an operator-independent, FDA-approved method to measure the endothelium-dependent dilation in response to reactive hyperemia (10). The PAT device records digital pulse wave amplitude using fingertip plethysmography (EndoPAT; Itamar Medical Ltd, Caesarea, Israel) and quantifies the endothelium-mediated changes in vascular tone, elicited by a 5-minute occlusion of the brachial artery. A post-occlusion to pre-occlusion ratio (**Fig. 2**) is calculated by the EndoPAT^{TM} software and expressed as RHI; **Fig. 2a**) or its natural logarithm variant (LnRHI; **Fig. 2b**). These values are normalized to measurements from the contra-lateral arm, which serves as control for non-endothelial dependent systemic effects. An RHI value less than 1.67 or an LnRHI value less than 0.51 denotes an endothelial dysfunction (8). EndoPAT was found to be well correlated with coronary endothelial function, evaluated by quantitative coronary angiography after injection of acetylcholine (8), as well as with the conventional cardiovascular risk factors (17,18). EndoPAT measurement was able to identify patients with early coronary atherosclerosis (8) and predicts late cardiovascular adverse events (10). EndoPAT reliability was tested in healthy adults and adolescents and it was shown to be highly reproducible across intervals of 1 day (19) and 1 week or longer (20-22). Description of technical procedure is reported in the Suppl. Appendix.

Methodological advantages of this technology are: 1) simultaneous recording from both arms with an intra-subject control; 2) live assessment of occlusion and provocation quality in order

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to avoid incomplete occlusion during the exam; 3) large dynamic range of measurements due to the finger ability to vary local vascular tone; 4) operator independency as all analyses are automated. Limitations is this assessment include: 1) the assessment of endothelial function at a microcirculation level instead of at medium to large size vessel such as the brachial artery, as assessed via standard flow mediated dilatation 2) the use of a fixed time frame during the hyperaemic response to calculate the EndoScore, while the maximal hyperaemic response can occur with different delays for each patient, especially in older subjects (23); 3) the influence of autonomous nervous system or the temperature study room on finger vascular tone; 4) limited knowledge about the effect of circadian variation on RHI result.

Flow Mediated Dilation.

Flow Mediated Dilation (FMD) of brachial artery is a non-invasive technique widely used to assess endothelial function. The FMD result is reported as a percentage change between the peak diameter in response to reactive hyperaemia, after 5 minutes of artery occlusion, and the baseline diameter using the following equation:

FMD (%) = (Peak diameter – Baseline diameter)/Baseline diameter * 100

Moreover, the FMD evaluation after nitrate administration is able to differentiate the endothelium-independent vasodilation component due to alterations in smooth and not endothelial cell function, improving the endothelial function assessment. However, although the principle seems simple and many laboratories showed robust test-retest reliability for FMD under standardized conditions (24-26), appropriate high-resolution ultrasound equipment and highly skilled personnel are essential for obtaining an accurate and reliable measurement (18,20,27). Ghiadoni et al. evaluated the FMD twice in the same day and subsequently after 30 days after a dedicated training program in the setting of a multicenter evaluation of 135 healthy volunteers (27). FMD values did not differ over time and showed an intra-session coefficient of variation ranging from 7.6% to 11.9% and an inter-session

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coefficient of variation ranging from 11.6% to 16.1% across centres. No data has been however provided in CAD patients who may have higher intra and inter-session variability. Furthermore, it remains challenging to standardise FMD protocol and technical expertise across different sites. Therefore, we evaluated the endothelial function using the FMD jointly to the EndoPAT technology (**Fig. 3**), only in one study center– Bern University Hospital, Switzerland, with large prior experience with this methodology (28-31). Description of technical procedure is reported in the Suppl. Appendix.

Circulating biomarkers

Plasma levels of various circulating biomarkers have been associated with the presence of endothelial dysfunction. Endothelin-1 (ET-1) is a 21-amino-acid peptide synthesized from a larger preproET-1 precursor that elicits its vasoconstrictor properties binding two G-protein coupled receptors, ET_A and ET_B, located on vascular smooth muscle cells, fibroblasts, and endothelial cells (ET_B only). ET-1 induces endothelial dysfunction reducing NO bioavailability through 2 different ways: 1) Decreasing its production via caveolin-1-mediated inhibition of eNOS activity; 2) Increasing its degradation via formation of oxygen radicals (32). Accordingly, high levels of ET-1 may represent an indirect index of endothelial dysfunction.

Asymmetric dimethylarginine (ADMA) is an analogue of L-arginine found in human circulation and represents a naturally occurring endogenous inhibitor of NO synthase (33). Reducing NO production, ADMA could thus lead to endothelial dysfunction and cardiovascular events. Elevated plasma ADMA concentration has been identified as an independent risk factor for progression of atherosclerosis, cardiovascular death, and all-cause mortality (34-36).

Von Willebrand factor (vWF) is a multimeric glycoprotein synthesised exclusively in endothelial cells and megakaryocytes and released when endothelial cells are damaged. Thus,

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high level of circulating vWF may reflect endothelium damage or endothelial dysfunction. The close association between vWF and thrombogenensis or atherogenesis also suggests that high vWF levels may be a useful indirect indicator of atherosclerosis and/or thrombosis (37).

Blood collection was to be performed after EndoPAT assessment at each time point, using the control arm as sampling site for the quantification of ADMA, vWF antigen, and ET-1. To assess the relationship between residual platelet reactivity or percentage inhibition and effect of $P2Y_{12}$ oral blocker on endothelial function, a platelet function testing was also to be carried out using the VerifyNow system using both $P2Y_{12}$ and aspirin assays.

Markers of thrombin activity such as prothrombin fragment 1+2, fibrinopeptide A, and thrombin-antithrombin complex (TAT) were also measured for exploratory purpose. All circulating biomarkers reported above were predefined and will be analysed by a centralized laboratory (Istituti Clinici Scientifici Maugeri - IRCCS Lumezzane, Brescia, Italy). C-reactive protein will be measured with an immunoturbidimetric assay, while all the other biomarkers will be measured using commercially available ELISA tests (**Suppl. Tab. 2)**, including the vWF antigen, for which the ELISA test is a well validated method of measurement (38-40). Finally, to provide mechanistic data on how ticagrelor may be associated with improved endothelial function, adenosine plasma concentration (APC), ticagrelor and its metabolite AR-C124910XX will be dosed in two different reference laboratories (Q&Q labs AB, Bio VentureHub, Mölndal, Sweden, and Bioanalytical Covance Laboratory, Indianapolis, United States, respectively). An overview of all investigated biomarkers with the corresponding collection time points are shown in **Suppl. Tab. 3**. Protocols of sample collection, centrifugation, and storage were standardized in all sites (pre-analytical standardization) and are reported in **Suppl. Tab. 4**. When immediately frozen at -20°C (as stored in Amsterdam, Barcelona, Pavia, and Bern), or -80°C (as stored in Rotterdam), after centrifugation and never thawed, all assessed biomarkers are known to be stable in vitro for a long time (at least few years). The only exception is represented by adenosine, which is immediately degraded after

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blood collection with a half-life of a few seconds. In order to improve adenosine stability and allow its subsequent dosage, a stopping solution was immediately added to blood tube during sampling.

Collection of Adenosine samples.

Adenosine plasma samples were collected as described by Bonello et al (12). Venous blood (2.6 ml) was withdrawn under vacuum together with a STOP solution previously placed in S-Monovette® 2.6 ml, K3 EDTA (Sarstedt, Nümbrecht, Germany). This method allows blood sample to be mixed rapidly with 4 mL of STOP solution, which prevents adenosine degradation and uptake. The STOP solution was composed of NBMPR 0.1 mml/L, dipyridamole 0.04 mmol/L, AMPCP 0.22 mmol/L, ethylene-diamine-tetraacetic acid disodium salt dehydrate 15 mmol/L, 5-iodotubericidin 0.1 mmol/L, and erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA) 0.1 mmol/L dissolved in PBS, pH 7.4, 0.01 mmol/L (**Suppl. Tab. 5**). The sample with the STOP solution was centrifuged at 1640xg for 10 minutes at room temperature. The plasma supernatant was then transferred in a dedicated micro-tube and then stored at -20°C or, preferably, -80°C.

Randomization

Allocation of study treatment was performed via a Web-based interactive randomization system available at https://trials.advicepharma.com/hitech. Randomization was achieved with computer-generated random sequence with a random block size (from X to Y) stratified according to the clinical site and the presence of diabetes mellitus.

Treatment protocol and Follow-up

All patients received DAPT for the entire duration of the study, which included aspirin (75-160 mg/day orally) and, in random order, clopidogrel (600 mg orally as LD followed by 75

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mg/day) for 30±5 days, prasugrel (60 mg orally as LD followed by 10 mg/day, or 5 mg/day if age ≥ 75 years and/or weight ≤ 60 Kg) for 30±5 days, and ticagrelor (180 mg orally as LD followed by 90 mg twice a day) for 30±5 days. Follow-up visit schedule is shown in **figure 1**.

Study endpoints

The primary objective of the study is RHI at treatment steady state (i.e. after 30±5 days of treatment), evaluated with EndoPAT system and assessed 1-2 hour(s) after intake of the daily (for clopidogrel or prasugrel) or morning (for ticagrelor) maintenance dose of each investigated $P2Y_{12}$ inhibitor. The primary endpoint consists of two main comparisons: ticagrelor vs. prasugrel difference in RHI, and ticagrelor vs. clopidogrel difference in RHI. The secondary objectives include RHI 1-2 hour(s) after $P2Y_{12}$ inhibitor loading dose or prior MD administration and other biomarkers of endothelial function. Each of these secondary endpoints encompasses two main comparisons: ticagrelor vs. prasugrel difference, and ticagrelor vs. clopidogrel difference. The comparison between prasugrel and clopidogrel in terms of RHI and circulating biomarkers will be also reported for exploratory purposes.

Statistical considerations

The null hypothesis (H0) of this study is that the primary endpoint (difference in RHI at treatment steady state) does not differ during ticagrelor treatment as compared with prasugrel or clopidogrel. The alternative hypothesis (H1) is that the primary endpoint differs after ticagrelor as compared to prasugrel or clopidogrel. Sample size calculation was based on repeated two-way ANOVA (20), setting mean RHI at 1.8 with a within subjects standard deviation (SD) of 0.31. Hence, 36 patients completing all sequences (i.e. 6 patients/sequence) provides 90% power to detect a 10% RHI relative change in ticagrelor group with a two-sided alpha level at 5%. To account for dropouts as well as incomplete data assessments, the final sample size was increased up to ≥50 patients.

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The primary endpoint will be analysed using repeated measures one-factorial analysis of variance (ANOVA with 3 levels as treatment factor to account for each of the three tested $P2Y_{12}$ inhibitor). Correction for possible intra-group correlation will be done by the Greenhouse-Geisser method. The ANOVA will yield the differences between the two main comparisons ticagrelor RHI vs. prasugrel RHI and ticagrelor RHI vs. clopidogrel RHI. To assess the primary endpoint, the significance of these two main comparisons will be combined using the Hochberg-Benjamini method (41) as follows: the H0 of randomized treatment equivalence comparing the response in RHI after ticagrelor vs. prasugrel administration, and the response in RHI after ticagrelor vs. clopidogrel administration, is rejected if significance is achieved for both main comparisons at a two-sided alpha level of 0.05 (i.e. the difference in RHI ticagrelor vs. prasugrel is supported with p-value < 0.05 and the difference in RHI ticagrelor vs. clopidogrel is supported with p-value < 0.05); or for one comparison at a two-sided alpha level of 0.025 (i.e. the difference in RHI ticagrelor vs. prasugrel is supported with p-value < 0.025 or the difference in RHI ticagrelor vs. clopidogrel is supported with p-value < 0.025).

Each of the secondary endpoints will be analysed using the same pre-defined statistical assumptions, combining the p-values of the two main comparisons. The H0 will be rejected if either comparison yields a p-value < 0.025 or when both comparisons yield p-values < 0.05. See Suppl. appendix for further explanation and for examples regarding the application of the Benjamini-Hochberg in our experimental setting (**Suppl. Table 6**).

The third comparison (i.e. the difference in response between prasugrel and clopidogrel) will be reported as explorative unpowered endpoint.

The SWAP-2 study showed that switching over to prasugrel from previous treatment with ticagrelor results in a progressive decline in PLT inhibition (leading to a clear rebound effect if no LD is given) as compared to the continuation of treatment with ticagrelor. Hence, it may be assumed that any measurement performed during the early phase of any switch from one to

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another P2Y12 inhibitor may be confounded (i.e. it may also at least partially reflect prior exposure to the earlier P2Y12 inhibitor). Based on the timing of blood sampling in this study, one may assume this carry-over effect to disappear from after 48 hours and up to 7 days. This was the rational for setting the primary EP measure remotely, i.e. at 30 days after each cross over. Nevertheless, methodologies that account for any possible carry-over effect, such as ANCOVA and stratified analysis based on the randomized sequence as well as non-randomized type of $P2Y_{12}$ inhibitor before randomization, will be applied for multiple sensitivity analyses for all primary and secondary endpoints. It is also pre-specified that for these sensitivity analyses, clopidogrel and prasugrel will be handled separately as well as lumped together in the thienopyridines group.

Stratified analysis of the primary endpoint will be also carried out according to: sex, age, presence of diabetes, hypertension, active smoking, dyslipidaemia, BMI, study site, number of vessels diseased, type of ACS at presentation, randomization sequence, and pre-randomization $P2Y_{12}$ inhibitor intake.

Predefined sub-analyses and sub-studies

Pre-specified sub-analyses and sub-studies include, but are not limited to, the evaluation of adenosine plasma concentration, adherence to study drugs, effect of age or concomitant drugs on primary or secondary endpoint measures of endothelial function and FMD results.

Study organization

The HI-TECH study was conducted at 5 investigative sites in 4 European countries, including Switzerland, Italy, Spain, and The Netherlands. The final study protocol and informed consent have been reviewed and approved by the ethics boards/institutional review boards and corresponding health authorities for all participant study sites/countries. The study is an investigator-driven clinical trial partially supported by an unrestricted research grant from

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AstraZeneca. The authors are solely responsible for the design and conduct of this study; all study analyses, and drafting and editing of the manuscript. Data are being coordinated and analysed by an academic Clinical Trial Unit (CTU) located in Bern, Switzerland. The trial registration number is NCT02587260 available at https://register.clinicaltrials.gov/prs/app/action/LoginUser?uid=U0002SC5&ts=167&cx=-f91kzu. Independent study monitoring was performed by AdvicePharma (Milan, Italy). The Electronic Data Capture (EDC) was designed by the investigators and web-implemented by AdvicePharma.

Conclusions

The HI-TECH study is the first randomized, cross over study aiming to ascertain whether ticagrelor, when administered at approved regimen in post-ACS patients, improves endothelial function as compared with both clopidogrel and prasugrel. The first patient was randomized on December 2015 and the last on October 2016 with a total of 54 patients. The last follow-up visit was performed on February 2017 and the final results are expected in Q3 2017.

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Conflicts of interest:

Salvatore Brugaletta received lectures fees from AstraZeneca, Abbott, and Boston, and has received institutional research grant from AstraZeneca. Sergio Leonardi received personal fees from AstraZeneca, The Medicine Company, Merck, Daiichi Sankyo, Ely Lilly and institutional research grants from AstraZeneca and Daiichi Sankyo. K. Stephan Windecker received research contracts to the institution from Abbott, Biotronik, Boston Scientifc, Medtronic, Edwards Lifesciences and St Jude. Marco Valgimigli has received speakers fees

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from AstraZeneca, Biosensor, and Terumo, and has received institutional research grants from AstraZeneca, The Medicines Company, and Terumo. Other authors have nothing to declare.

Contributorship:

MV designed the study and obtained funding; MV and SA contributed to protocol development; MV and SA actively participated in the writing of the manuscript; DH contributed to the statistical analysis and actively participated in the writing of the statistical sections of the manuscript; all authors critically reviewed the manuscript and approved the final version.

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Figure legend

Figure 1. Study design

Study flow-chart depicting study visits with the respective time intervals (black arrows on top) and all procedures performed at each time-point. Endothelial function evaluation is performed using EndoPAT in all sites while FMD was performed only for patients recruited in Bern. Blood was collected for dosage of circulating biomarkers. VerifyNow measurements, using both $P2Y_{12}$ and ASA assays were obtained to assess platelet reactivity. ACS=Acute Coronary Syndrome; V1=visit one; V2=visit two; V3=visit three; V4=visit four; V5=visit five; V6=visit six; LD=loading dose; MD=maintenance dose; T1=1-2 hours after ticagrelor LD administration; T2=before ticagrelor MD administration; T3=1-2 hours after ticagrelor MD administration; P1=1-2 hours after prasugrel LD administration; P2=before prasugrel MD administration; P3=1-2 hours after prasugrel MD administration; C1=1-2 hours after clopidogrel LD administration; C2=before clopidogrel MD administration; C3=1-2 hours after clopidogrel MD administration; S=sequence; P=prasugrel; T=ticagrelor; C=clopidogrel; h=hour(s).

Figure 2. EndoPAT™ Measurement

Figure 2a. Reactive hyperemia index (RHI) measurement after EndoPAT™ assessment. A=mean PAT amplitude between 90s-150s post-occlusion of the test arm; B=mean PAT amplitude from the baseline period on the test arm; C=mean PAT amplitude between 90s-150s post-occlusion of the control arm; D=mean PAT amplitude from the baseline period of the control arm.

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Figure 2b. Natural logarithm of Reactive Hyperemia Index (LnRHI) measurement after EndoPAT^{TM} assessment. A=mean PAT amplitude between 90s-120s post-occlusion of the test arm; B=mean PAT amplitude from the baseline period on the test arm; C=mean PAT amplitude between 90s-120s post-occlusion of the control arm; D=mean PAT amplitude from the baseline period of the control arm.

Figure 3. FMD and EndoPAT assessment at Bern University Hospital.

The picture shows the concomitant acquisition of FMD and EndoPAT measurements at Bern University Hospital.

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Table 1. Inclusion and exclusion criteria

Inclusion Criteria
1. Age > 18 years
2. ACS (including STEMI or NSTEMI) at least 30 days before randomization
3. Ongoing treatment with DAPT since at least 30 days, consisting of ASA 75-160 mg daily and one of the three available $P2Y_{12}$ inhibitors (ticagrelor, prasugrel or clopidogrel)
4. No bleeding events (defined as BARC type 2 or greater) or ischemic recurrences in the period between the ACS and the study randomization
Exclusion Criteria
1. Administration of fibrinolytics or glycoprotein IIb/IIIa inhibitors in the previous 30 days
2. Major surgery within 30 days or any planned surgical or percutaneous intervention
3. Active bleeding or previous clinically relevant bleeding in the last 6 months
4. Previous TIA or stroke in the last 6 months
5. Previous intracranial bleeding
6. Thrombocytopenia
7. Ongoing anticoagulant therapy or clinical indication to start with anticoagulant agents
8. Vasculitis or any known immunological disorder
9. Severe hepatic failure
10. Uncontrolled hypertension (systolic or diastolic arterial pressure > 180 mmHg or 120 mmHg, respectively, despite medical therapy)
11. Known intolerance to aspirin, clopidogrel, prasugrel, or ticagrelor
12. Limited life expectancy (i.e. neoplasms)
13. Inability to obtain the informed consent
14. Pregnancy

ACS=acute coronary syndrome; STEMI=ST segment elevation myocardial infarction; NSTEMI=non-ST segment elevation myocardial infarction; DAPT=dual antiplatelet therapy; ASA=aspirin; BARC=bleeding academic research consortium; TIA=transient ischemic attack

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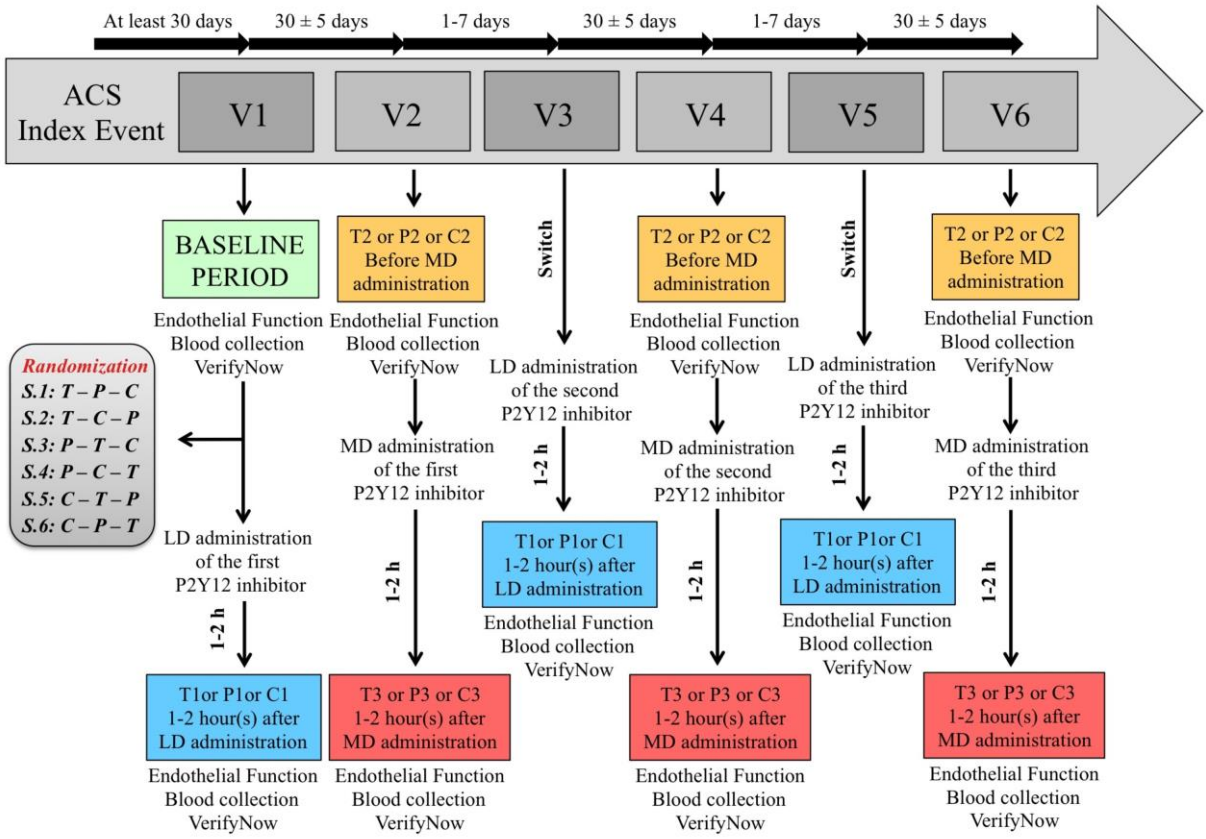

Figure 1

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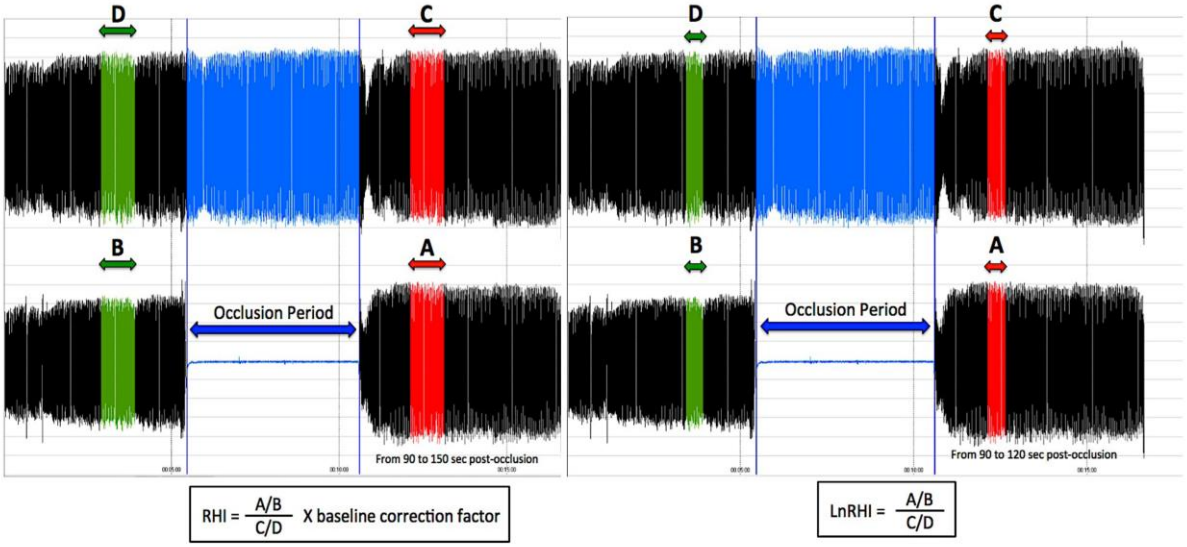

Figure 2

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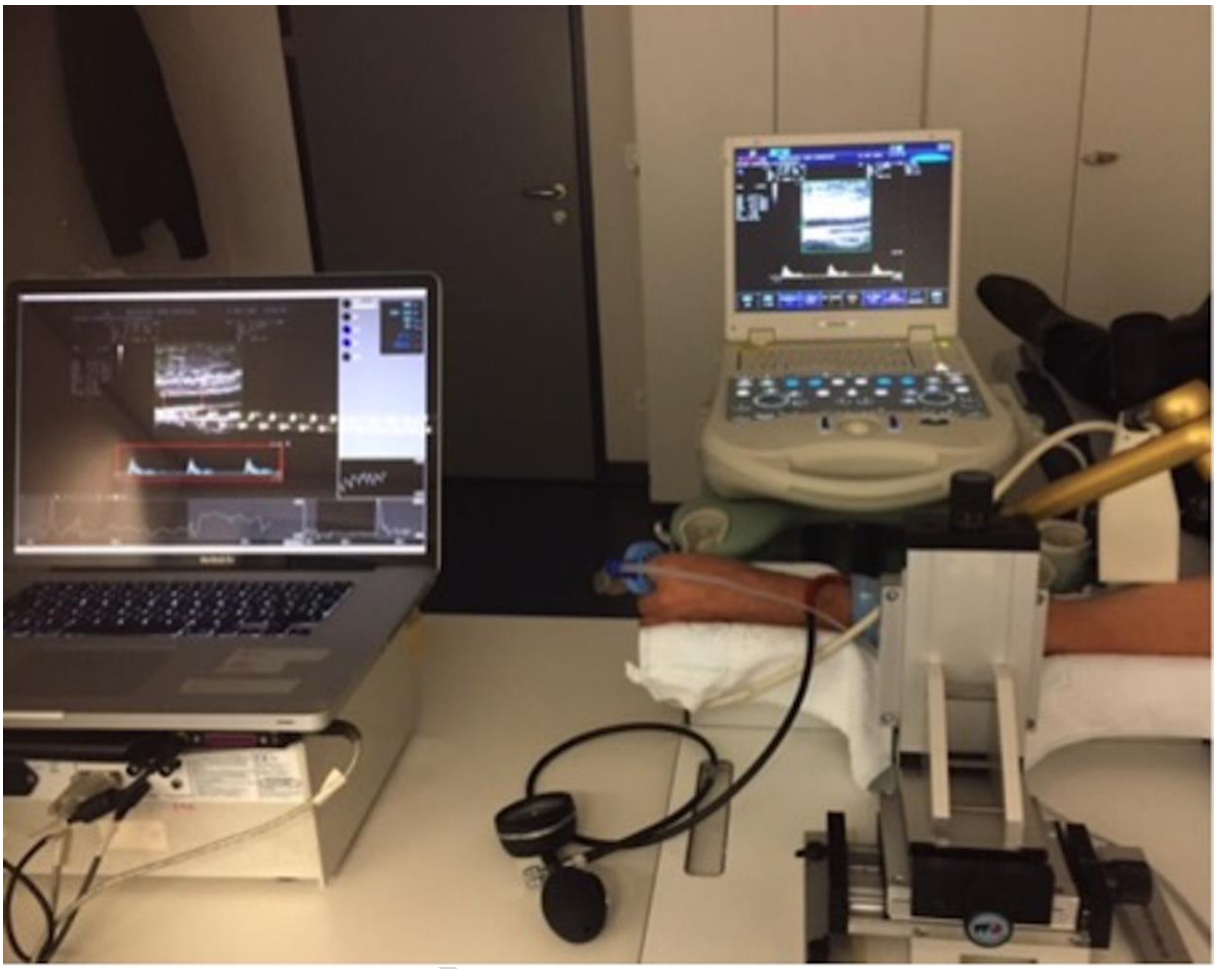

Figure 3

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