Supplementary Material

Effects of ticagrelor and clopidogrel on coronary microcirculation in patients with acute myocardial infarction

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Inclusion criteria

a) Age > 18 years and ≤ 75 years;
b) Patients diagnosed with STEMI up to 24 hours in duration, documented by ischemic symptoms due to atherosclerosis of > 10 minutes at rest, treated with pharmacological thrombolysis, ASA and anti-ADP.
c) Cardiac catheterization performed within 4 (± 2) days from the onset of symptoms and with residual obstruction in the "guilty" artery <50% with TIMI 3 flow at coronary angiography, regardless of whether or not the patient underwent percutaneous coronary intervention.
d) Agreement to sign the informed consent form

Exclusion Criteria

a) Previous infarction on the same wall as the current one.
b) Any contraindication to the use of clopidogrel or ticagrelor according to the product instructions (eg, hypersensitivity, moderate to severe liver disease, active bleeding or recent bleeding history, history of intracranial hemorrhage).
c) Need for oral anticoagulation therapy or ASA doses greater than 100mg / day.
d) Concomitant oral or IV therapy with strong CYP3A inhibitors (ketoconazole, itraconazole, voriconazole, telithromycin, clarithromycin, nefazodone, ritonavir, saquinavir, nelfinavir, indinavir, atazanavir, N1 L / dP grapefruit juice, substrates of C3P indexes with therapeutic substrates narrow (cyclosporine, quinidine), or strong CYP3A inducers (rifampicin, phenytoin, carbamazepine).
e) High risk of bradyarrhythmias.
f) Need for dialysis.
g) Known thrombocytopenia clinically important.
h) Clinically important anemia.
i) Any other condition that could put the patient at risk or influence the results of the study in the investigator's opinion (eg, cardiogenic shock, severe hemodynamic instability, active cancer).
j) Pregnancy or lactation.
k) Any condition that increases the risk of non-adherence or loss of follow-up.
I) Contraindications to fibrinolytic therapy including: any previous intracranial hemorrhage or known structural cerebral vascular injury (eg, arterial venous malformation) or known malignant intracranial neoplasia (primary or metastatic) or ischemic stroke within 3 months or suspected aortic dissection or active bleeding or bleeding diathesis (excluding menstruation) or head trauma or significant facial trauma within 3 months.

m) Participation in another study.

Study design
Following is the temporal summary of the study design:

Supplementary Figure S1

Supplemental Figure S1

MCE (Myocardial contrasted echocardiography)

Patient inclusion
Initially, patients from 2 centers participating in the TREAT study were screened, namely the General Hospital of Grajau of São Paulo / Brazil and the Hospital de emergencias Albert Sabin of São Caetano / Brazil. During the period from September 2016 to September 2017, 122 patients already randomized to ticagrelor or clopidogrel were transferred consecutively to the CCU of the Instituto
do Coração / InCor - HCFMUSP. Of these, 74 patients had some exclusion criteria and 48 patients were selected for the present study, 24 in the ticagrelor group and 24 in the clopidogrel group, were analyzed in the groups to which they were randomized ('intention to treat' analysis).

**Supplementary Figure S2**

![Patient inclusion flowchart](image)

**Pts (Patients); MCE (Myocardial contrasted echocardiography)**

**Echocardiography methods**

**Myocardial Contrasted Echocardiography (MCE)**

The Myocardial Perfusion Score Index (MPSI) and Parametric Image (PI) are MCE methods for analyzing myocardial perfusion and evaluating the microcirculation. The MPSI analysis is performed using the Gray Scale (GS), and the PI from which the Infarction Area Analysis (IAA) is obtained, is performed by
color coding, and both are displayed as a set of images of according to the relative degree of myocardial perfusion. (1) PI provides the area and transmural extension of the perfusion defect, that is, the infarcted area. Both echocardiographic analysis of quantitative myocardial perfusion using PI and GS were validated as a method for measuring the infarcted area in humans. (2)

To obtain the MCE images, the ultrasonic contrast agent SonoVue® (Bracco Imaging - Florence, Italy) was used, which was administered in bolus of 1mL in 1mL in order to maintain an optimal infusion rate for each patient, to maintain an ideal myocardial enhancement. The proper mixing of the solution was maintained by continuous and careful stirring of the contrast-containing solution. All patients underwent the imaging method using an ultrasound system (IE33, Philips Medical Systems - Bothell, USA) equipped with an S5-1 scanning head.

MCE images were acquired at rest using apical views of 4, 2 and 3 chambers, using the myocardial perfusion image modality in real time and low mechanical index (MI) (IM 0.1-0.2). The manual delivery of a 5-frame flash sequence with high MI (IM 1.4) was performed to destroy the microbubbles in the scanning plane and, therefore, allowing the visualization of subsequent myocardial replacement. The echocardiographic definitions were adjusted to optimize myocardial opacification and to minimize attenuation artifacts before each study and were kept constant throughout. All images were recorded on external HD for future analysis.

MCE data was transferred to an offline workstation for analysis (Q-Station 3.3.2, Philips Healthcare, Andover, MA). MPSI and PI were assessed using a myocardial segmental model in all 3 standardized views for MCE. For myocardial perfusion analysis, the moving images were evaluated according to the complete destruction of microbubbles in the image immediately after the flashes, clear myocardial filling and good image alignment with minimal artifacts caused by the patient's breathing, shading and the evaluator's movement. These images were selected for MPSI and PI analysis.

Evaluation of the Myocardial Perfusion Score Index (MPSI)
For the analysis of MPSI by MCE, the left ventricle was divided into 17 segments. Each segment was graded with a Perfusion Score (PS), where PS = 1 representing normal opacification, PS = 2 representing reduced opacification and PS = 3 representing absence of opacification. In case of visual limitations of a given segment, it was considered PS = 0. The Global Myocardial Perfusion Score Index (Global MPSI) was then obtained by adding the PS of each segment divided by the number of total segments (17 segments). The Regional Myocardial Perfusion Score Index (Regional MPSI) was obtained by adding the PS of each segment related to the artery responsible for the infarction (Anterior and Non-Anterior Segments). No-reflow patients were defined as those with PS = 3 in > 2 segments. (3,4)

**Parametric image (PI) and the Infarction Area Analysis (IAA)**

After obtaining the images, as previously described to generate the PI, an assisted edge detection algorithm was used to segment the myocardium from the rest of the image. A color map was applied to generate PI blood volume ($\alpha$), blood flow rate ($\beta$) and myocardial blood flow ($\alpha \times \beta$) for each pixel in the myocardium. The values of each pixel were color-coded in relation to the average value. Color gradations were applied linearly based on the fractions of the mean value, with break points fixed in the mean (green), two-thirds of the mean (yellow) and one-third of the mean (red), corresponding to normal myocardial perfusion, moderate reduction and severe, respectively. Myocardial infarction was defined by PI as the red area in regions with abnormalities of wall movement using blood flow velocity ($\beta$), while by PS it was defined as the area without opacification in regions with abnormalities in wall movement. PI was used to calculate the infarction area and the blood flow velocity ($\beta$) was chosen to calculate the infarcted area because it showed a better correlation with the magnetic resonance data.(2) The final systolic picture of each segment and its area of infarction were measured by planimetry using software PI (Image J [cm2], National Institutes of Health, Bethesda, MD). The total area of the myocardium was derived by the total count of each of the 17 measured myocardial segments and the area of infarction was derived from the count of each infarcted area of the segment. All images were
analyzed by an experienced observer blinded to the clinical data of the intervention. The size of the infarction was classified as small when less than 19% of the entire area of the myocardium, medium when it was between 19% and 30% and large when greater than 30% of the entire area of the myocardium.

**Evaluation of the Wall Motion Score Index (WMSI)**

As in the perfusion assessment, to assess contractility, each segment was graded with a Montion Score (MS), where MS = 1 represents normal contractility, MS = 2 represents reduced contractility and MS = 3 represents absence of contractility. In case of visual limitations of a particular segment, it will receive MS = 0. The Global Wall Motion Score Index (Global WMSI) was then obtained by adding the MS of each segment divided by the number of total segments (17 segments). The Regional Wall Motion Score Index (Regional WMSI) was obtained by adding the MS of each segment related to the artery responsible for the infarction (Anterior and Non-Anterior Segments)(3,4)

In the sequence (Figure S3), image of the MCE showing the analysis of the MPSI by the CS (Plan A) and analysis of the IAA by the PI (Plan B):

**Supplementary Figure S3**

Myocardial Contrasted Echocardiography
A = Left-ventricle image in 2-chamber view in a patient with STEMI of the anterior wall in gray scale for obtaining the MPSI. There is a perfusion defect in the Apical segment.
B = From Image A, evaluation by PI (β value) in color, to obtain the IAA. An area with no perfusion is observed in red, representing the infarction area. An area with decreased perfusion in yellow is observed.

STEMI = Myocardial Infarction with ST-segment elevation; MPSI = Myocardial Perfusion Score Index; PI = Parametric Image; AAI = Infarction Area Analysis.

**Platelet aggregability**

The method used for platelet aggregability assessment was Multiplate®. No other tests regarding ADP activation nor ASPI activation were performed. Multiplate® (Multiple Electrode Aggregometry - Roche): test that analyzes the platelet function in whole blood at 37 ° C by connecting the platelets to metal electrodes, leading to changes in electrical conductance (or impedance), after activation of the same via ADP receivers by the ADP-Test. The results are obtained in aggregation speed or area under the curve (AUC) and, the higher, the greater the platelet aggregability. (5) Since it’s an Acute Myocardial Infarction trial, the patients were not fasting.
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