Original Manuscript

Ticagrelor Loading on ST-Elevation Myocardial Infarction: Interaction With Prodromal Angina on Infarct Size and Clinical Events

João Pedro Faria, MD[1,2] 📵, Pedro Oliveira, PhD[2,3], André Alexandre, MD[4] 📵, David Sá Couto, MD[4], Ricardo Costa, MD[4], Andreia Campinas, MD[4], André Frias, MD[4], Bruno Brochado, MD[4], Raquel Santos, MD[4], João Silveira, MD[2,4], Severo Torres, MD[2,4], and André Luz, MD, PhD, FESC[1,2,4]

Abstract

Introduction: Ticagrelor might reduce infarct size by exerting a more potent antiplatelet effect or by promoting a potential conditioning stimulus in ST-elevation myocardial infarction (STEMI) patients. Pre-infarction angina (PIA) is an effective preconditioning stimulus that reduces ischemia-reperfusion injury. Because little is known on the interaction of PIA in STEMI-patients loaded with ticagrelor, we sought to determine if patients loaded with ticagrelor had improved clinical outcomes as compared to clopidogrel and to study if it is modulated by the presence of PIA. **Methods:** From 1272 STEMI patients submitted to primary percutaneous coronary intervention and treated with clopidogrel or ticagrelor from January 2008 to December 2018, 826 were analyzed after propensity score matching. Infarct size was estimated using peak creatine kinase (CK) and troponin T (TnT), and clinical impact was evaluated through cumulative major cardiac and cerebrovascular events (MACCE) at 1-year follow-up. Matched patients and their interaction with PIA were analyzed. **Results:** Patients loaded with ticagrelor had lower peak CK [1405.50 U/L (730.25-2491.00), $P < .001$] and TnT [3.58 ng/mL (1.73-6.59), $P < .001$], regardless of PIA. The presence of PIA was associated with lower CK ($P = .030$), but not TnT ($P = .097$). There was no interaction between ticagrelor loading and PIA ($P = .788$ for TnT and $P = .555$ for CK). There was no difference in MACCE incidence between clopidogrel or ticagrelor loading ($P = .129$). Cumulative survival was also similar between clopidogrel or ticagrelor, regardless of PIA ($P = .103$). **Conclusion:** Ticagrelor reduced infarct sizes independently and without a synergic effect with PIA. Despite reducing infarct size, clinical outcomes were similar across both groups.

Keywords

ticagrelor, cardioprotection, conditioning, STEMI, pre-infarction angina

Introduction

In patients presenting with ST-elevation myocardial infarction (STEMI), the best evidence-based approach in reducing infarct size and mortality is timely primary percutaneous coronary intervention (PPCI).[1] However, despite rescuing the ischemic cardiomyocytes from further infarction, reperfusion is also associated with an additional component of irreversible myocardial damage called the ischemia-reperfusion (IR) injury phenomenon.[2-6] While several cardioprotective strategies have consistently shown considerable promise in reducing the IR injury in preclinical studies, their translation to clinical practice has been unsatisfactory.[7-10]

Potent oral antiplatelet agents have been a mainstay of current PPCI to limit clot organization and to prevent stent

[1] Cardiovascular Research, UMIB—Unit for Multidisciplinary Research in Biomedicine, University of Porto, Porto, Portugal
[2] ICBAS—School of Medicine and Biomedical Sciences, University of Porto, Porto, Portugal
[3] ISPUP-EPIUnit—Institute of Public Health of University of Porto, Porto, Portugal
[4] Department of Cardiology, Centro Hospitalar Universitário do Porto (CHUPorto), Porto, Portugal

Corresponding Author:
João Pedro Faria, ICBAS—School of Medicine and Biomedical Sciences, University of Porto, Rua de Jorge Viterbo Ferreira n°228, 4050-313 Porto, Portugal.
Email: jpff@live.com.pt

thrombosis. Ticagrelor, a P2Y$_{12}$ inhibitor, has been used for this purpose in patients presenting with acute coronary syndrome (ACS).[1] It has been suggested that ticagrelor has pleiotropic effects, exerting a potential conditioning stimulus through the inhibition of the Equilibrative Nucleoside Transporter 1 (ENT-1), expressed on the surface of erythrocytes, which leads to reduced adenosine reuptake.[11-13] Adenosine would then act on cardiomyocytes, activating multiple pathways, including the Reperfusion Injury Salvage Kinase (RISK) and Survival Activating Factor Enhancement (SAFE) signaling pathways.[2,3] These are thought to promote the survival of the cardiomyocyte by inhibiting the mitochondrial permeability transition pore (MPTP) and stimulating the expression of cardioprotective genes.[2,3] However, there are inconsistent data regarding the potential cardioprotective effect of ticagrelor, with conflicting results.[14-20]

On the other hand, and despite not being entirely consensual, the presence of pre-infarction angina (PIA), which is believed to be a very potent conditioning stimulus protecting the cardiomyocyte from the IR injury,[3] has been most consistently shown to limit infarct size, reduce mortality, and improve left ventricle ejection fraction (LVEF) in patients presenting with STEMI.[2,21-28] Therefore, it is possible that PIA may render additional cardioprotective agents irrelevant.

While several studies have researched the individual cardioprotective effect of ticagrelor and PIA, to the best of our knowledge, the potential synergism between PIA and ticagrelor has not yet been studied. Given the unmet need in identifying a strategy to limit the IR phenomenon, and postulating that ticagrelor loading in an already preconditioned patient might hamper any conditioning maneuver at reperfusion, our aim was to determine if ticagrelor loading in STEMI patients with or without preexisting PIA is related to improved outcomes.

Materials and Methods

Study Population and Definitions

We carried out a retrospective, observational, cohort study which included every consecutive patient with an admission for a STEMI who underwent PPCI, with a clearly identifiable culprit lesion, between January 1st 2008 and December 31st 2018, at a tertiary hospital. All patients were loaded with aspirin (500 mg) and either clopidogrel (600 mg) or ticagrelor (180 mg) before PPCI (given in ambulance or in the emergency room upon arrival). Only naïve patients to clopidogrel or ticagrelor were included in the analysis. The myocardial necrosis biomarkers were serially sampled by local protocol upon admission, then at 6 h, 12 h, 24 h and 48 h. We excluded patients who were not treated with ticagrelor or clopidogrel at admission and those that switched the P2Y$_{12}$ inhibitor during hospital stay or at follow-up. We also excluded patients who presented with a culprit lesion at left main coronary artery or at a previous coronary bypass graft, and also those who came intubated and hence a loading dose was not given before catheterization. For patients with multiple STEMI-related

admissions, we only considered the first admission during the study period, with the remaining admissions being considered on follow-up. The diagnosis of STEMI was established according to the criteria defined by the European Society of Cardiology.[1] The clinical, demographic, laboratory and procedural data for these patients were retrospectively obtained by electronic medical chart review and included in an anonymized database. PPCI was performed mostly by radial approach. As per local protocol, the only anticoagulant agent given was unfractionated heparin (70 Units/Kg) by a peripheral vein to all patients undergoing PPCI. IIb/IIIa inhibitors were administered at the discretion of the interventional cardiologist. After completion of PPCI, all patients were admitted to the coronary care unit where evidence-based care was rendered as per current guidelines.

PIA was diagnosed at admission when a patient presented arm, jaw, or chest pain, at rest or during exercise, lasting less than 20 minutes, in the week before the STEMI diagnosis; this was systematically inquired to every patient, according to a prespecified checklist protocol at admission. Total ischemic time is defined as the time between symptom onset and the passage of coronary guidewire with restoration of blood flow.

We calculated the Thrombolysis in Myocardial Infarction (TIMI) score, which gives an estimate of mortality in patients with STEMI,[29] and the SYNTAX score, which estimates the complexity of multivessel coronary artery disease.[30] We stratified SYNTAX into 3 groups in order to have approximately a third of patients in each group: ≤16.00, 16.01-22.00 and ≥22.01. The estimated glomerular filtration rate (eGFR) was calculated according to the Cockcroft-Gault formula.

LVEF was calculated using 2D-echocardiography and stratified in 4 groups: good LVEF was considered for values equal to or higher than 55%; mild impairment was considered if LVEF was between 45% and 55%; moderate impairment if LVEF ranged from 35% and 45%; and severe impairment if LVEF lower than 35%.

The 1-year clinical follow-up data, regarding the occurrence of major cardiac and cerebrovascular events (MACCE) after the index STEMI, were obtained from electronic records by chart review. This study was approved by the ethics committee of Centro Hospitalar Universitário do Porto (2021.291[237-DEFI/245-CE]). Informed consents were waived due to the anonymization and retrospective nature of the study.

Definition of Clinical Outcomes

Infarct size was estimated using peak creatine kinase (CK) and peak troponin T (TnT) as surrogates from the samples drawn at 6, 12, 24 and 48 h. TnT concentration was measured by Elecsys Modular Analytics e170 (Roche Diagnostics, Mannheim, Germany); CK activity was measured on a Roche Cobas 8000 analyzer (c701 module; Roche Diagnostics). Cumulative MACCE at 1 year follow-up is a composite of death of any cause, stroke with imaging evidence, recurrent myocardial infarction (MI), and target lesion revascularization (TLR)

(defined as angina or restenosis with ischemia, requiring reintervention, of a previous culprit lesion).

Statistical Analysis

Continuous variables were expressed as mean $\pm$ standard deviation (SD) when they followed a normal distribution or as median and interquartile range, between quartiles 25 (Q1) and 75 (Q3), when non-normally distributed. Categorical variables were represented as absolute values and percentages [n (%)]. The normality of distribution was assessed by Shapiro-Wilk and Kolmogorov-Smirnov tests.

To compare continuous variables between groups, we used an independent Student *t* test when variables followed a normal distribution or Mann-Whitney *U* test for non-normally distributed variables. To compare categorical values, we used χ^2 or Fisher exact tests, when appropriate.

The logarithmic transformation was applied to peak CK, peak TnT, total ischemic time and SYNTAX score in order to allow for parametric testing and adequate propensity score (PS) matching.

As the standard of antiplatelet therapy changed during the study period, with ticagrelor being favored over clopidogrel, we attempted to reduce the treatment assignment bias by using PS to match each patient treated with ticagrelor to a comparable patient treated with clopidogrel. To estimate the PS, we used 7 baseline characteristics, namely sex (male/female), age (years), smoking habits (yes/no), diabetes mellitus (yes/no), dyslipidemia (yes/no), hypertension (yes/no), and eGFR (mL/min). We also used 6 variables related to procedural data, particularly TIMI and SYNTAX risk scores, anterior acute myocardial infarction (yes/no), LVEF at discharge (good/mild/moderate/severe), Killip class ($\leq 2/\geq 3$) and total ischemic time (minutes). The match tolerance was set at 0.01. We then performed all comparative analyses after PS matching.

To analyze the interaction between PIA and the use of ticagrelor or clopidogrel, a 2-way factorial ANOVA was computed.

We estimated cumulative survival at follow-up with a Kaplan-Meier analysis, using the log-rank test for comparisons.

For all tests, a two-sided *P* value less than .05 was considered statistically significant. We used SPSS version 27.0 (SPSS Inc., Chicago, IL, USA) for all comparative statistics and propensity score matching.

Results

Study Population

A total of 1272 STEMI patients were treated by PPCI between January 1st 2008 and December 31st 2018. Of these, 1214 patients (95%) fulfilled the entry criteria and comprised our study sample. Tables S1 and S2 show the demographic and procedural characteristics and in-hospital outcomes, respectively, in accordance with ticagrelor vs clopidogrel loading, prior to PS matching. Patients who received ticagrelor were

younger, had better renal function and were pre-treated more often with calcium channel blockers. Both groups presented similar procedural characteristics, except for door-to-balloon time, IIb/IIIa inhibitors and thrombectomy usage, and length of stay, which were lower on patients treated with ticagrelor. Furthermore, patients treated with clopidogrel had lower radial access and coronary disease complexity, as measured by the SYNTAX score. At discharge, patients treated with ticagrelor were less prescribed with β-blockers and angiotensin-converting enzyme inhibitors. LVEF was not different at discharge from hospital.

By applying the PS matching as explained in the previous section, 413 patients loaded with ticagrelor were matched with 413 patients loaded with clopidogrel (Figure 1). Table 1 shows the baseline and procedural characteristics, and Table 2 shows the in-hospital outcomes after PS matching. In patients treated with clopidogrel, although more patients [151 (36.7%)] presented PIA at admission, this difference was not statistically significant. The remaining baseline characteristics were identical between the studied groups. Regarding procedural characteristics, total ischemic time was comparable, whereas door-to-balloon time, IIb/IIIa inhibitors infusion, and length of stay were lower in patients loaded with ticagrelor. Moreover, radial access and coronary artery disease complexity (SYNTAX score) were lower in patients treated with clopidogrel. No follow-up data was available in 3 patients (2%).

Outcomes

Patients treated with ticagrelor had a lower peak CK and TnT [1405.50 U/L (730.25-2491.00) for CK, *P* < .001, and 3.58 ng/mL (1.73-6.59) for TnT, *P* < .001], regardless of PIA (*P* < .001 for CK and TnT without PIA; *P* = .009 for CK and *P* = .006 for TnT in patients presenting with PIA) (Table 3, Figure 2). The presence of PIA was associated with lower CK values (*P* = .030), but not TnT (*P* = .097). No interaction was found between ticagrelor vs clopidogrel loading and PIA (*P* = .788 for TnT and *P* = .555 for CK, Figures S1 and S2).

Regarding MACCE at 1-year follow-up (Table 3, Figure 3), while cumulative MACCE tended to be higher in patients treated with clopidogrel (13.3% vs 8.0% in patients loaded with ticagrelor), there was no statistically significant difference between clopidogrel or ticagrelor loading (*P* = .129). Cumulative survival was also similar between clopidogrel or ticagrelor, regardless of PIA (*P* = .103, Figure 4).

Discussion

In this study, we showed that ticagrelor led to lower peak CK and TnT when compared to clopidogrel, and this effect is independent of PIA, which reduces peak CK, but not peak TnT. At discharge, there was no difference in LVEF between both groups. Also, there was no difference in MACCE at 1-year follow-up.

It has been consistently shown that the larger the infarct size, the higher the incidence of death, heart failure (HF) and an
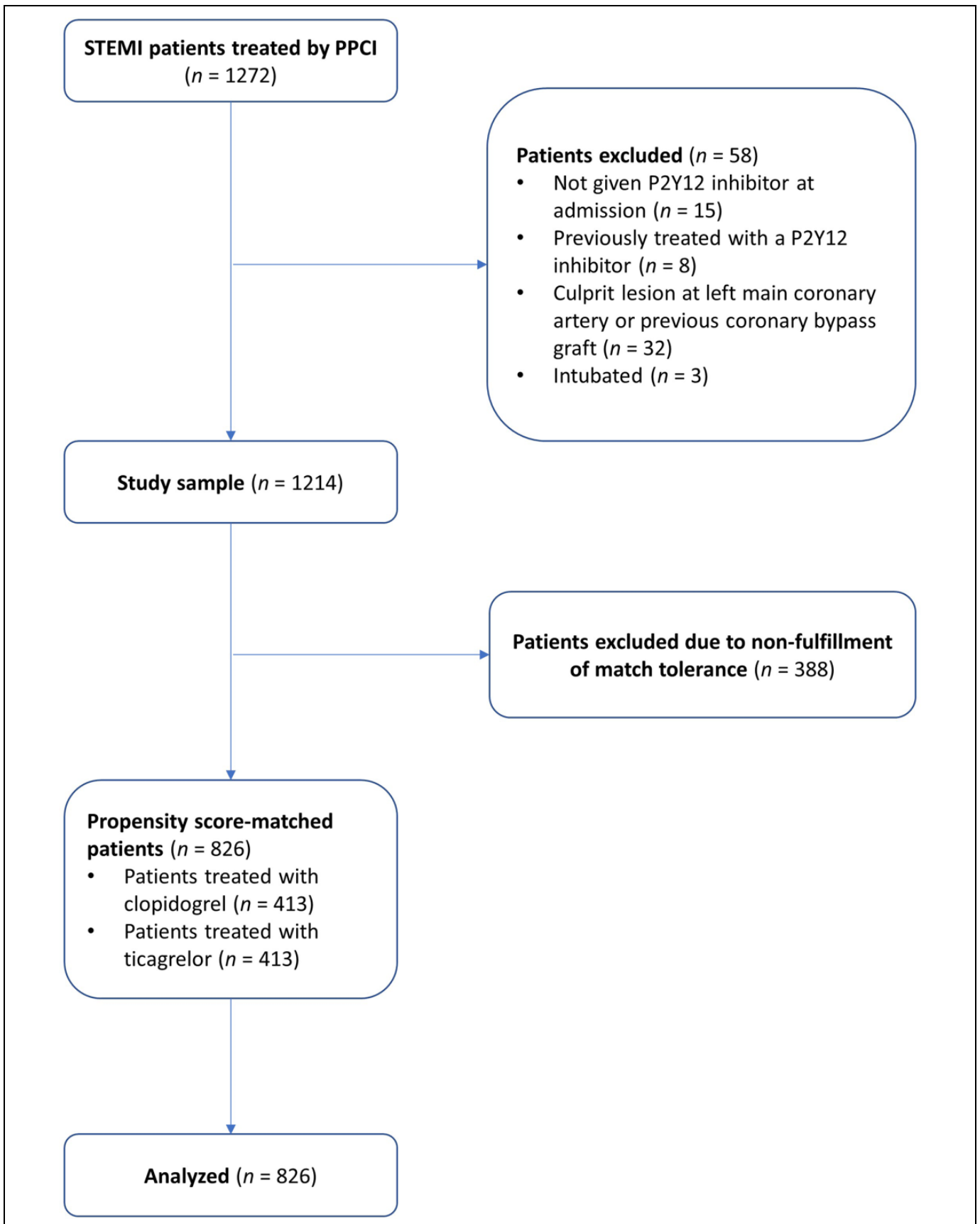
Figure 1. Flowchart of the study, illustrating the distribution according to propensity score matching. PPCI indicates primary percutaneous coronary intervention; STEMI, ST-elevation myocardial infarction.

Table 1. Baseline and Procedural Characteristics After Propensity Score Matching.

	Clopidogrel (n = 413)	Ticagrelor (n = 413)	P value
Baseline characteristics			
Age (years) (mean ± SD)	61.82 ± 13.37	61.96 ± 12.34	.877
Male [n (%)]	290 (70.2)	305 (73.8)	.245
BMI (kg/m^2) (mean ± SD)	26.43 ± 3.70	26.61 ± 4.36	.844
Diabetes mellitus [n (%)]	103 (24.9)	93 (22.5)	.511
Hypertension [n (%)]	240 (58.1)	228 (55.2)	.399
Dyslipidemia [n (%)]	230 (55.7)	214 (51.8)	.264
Active smoker [n (%)]	233 (56.4)	221 (53.5)	.401
Previous AMI [n (%)]	32 (7.8)	33 (8.0)	.897
Peripheral artery disease [n (%)]	34 (8.3)	32 (7.7)	.790
Family history of CAD [n (%)]	35 (13.4)	38 (9.2)	.087
Pre-infarction angina [n (%)]	151 (36.7)	125 (30.3)	.052
Creatinine (mg/dL) (Q1-Q3)	0.90 (0.74-1.08)	0.87 (0.73-1.03)	.146
eGFR (mL/min/1.73 m^2) (Q1-Q3)	87.00 (63.00-111.00)	86.80 (63.00-112.39)	.501
Hemoglobin at admission (g/dL) (mean ± SD)	14.09 ± 1.79	14.06 ± 1.70	.799
Ongoing medications [n (%)]			
β-blocker	47 (11.4)	55 (13.4)	.383
Statin	91 (22.0)	110 (26.8)	.109
ACE inhibitor	71 (17.2)	59 (14.4)	.264
Angiotensin receptor blocker	72 (17.5)	80 (19.5)	.452
Calcium channel blocker	42 (10.2)	59 (14.4)	.067
Aspirin	59 (14.3)	51 (12.4)	.420
Insulin	17 (4.1)	20 (4.9)	.614
Oral antidiabetics	72 (17.5)	62 (15.1)	.345
Nitrates	22 (5.4)	14 (3.4)	.173
Procedural characteristics			
Total ischemic time (minutes) (Q1-Q3)	240 (155.00-473.00)	240 (147.25-438.25)	.482
Door-to-balloon time (minutes) (Q1-Q3)	80.00 (54.50-127.50)	72.50 (44.00-120.00)	.023
Systolic BP (mmHg) (Q1-Q3)	121.00 (110.00-140.00)	120.00 (107.50-134.00)	.108
Use of IIb/IIIa inhibitors [n (%)]	123 (29.9)	40 (9.7)	<.001
Culprit artery [n (%)]			.830
LAD	176 (42.8)	179 (44.0)	
LCX	58 (14.1)	61 (15.0)	
RCA	177 (43.1)	167 (41.0)	
Thrombectomy utilization [n (%)]	306 (74.1)	142 (34.4)	<.001
Thrombectomy efficiency [n (%)]	272 (88.9)	122 (86.5)	.472
No reflow [n (%)]	23 (5.6)	24 (5.8)	.881
Radial access [n (%)]	184 (44.6)	385 (93.4)	<.001
Killip class [n (%)]			.816
≤2	371 (89.8)	373 (90.3)	
≥3	42 (10.2)	40 (9.7)	
TIMI score (Q1-Q3)	3.0 (2.0-5.0)	3.0 (2.0-5.0)	.706
Syntax score [n (%)]			.002
≤16.00	178 (43.1)	182 (44.1)	
16.01-22.00	123 (29.8)	84 (20.3)	
≥22.01	112 (27.1)	147 (35.6)	
Length of stay (days) (Q1-Q3)	6 (5-9)	6 (5-8)	.010
IABP [n (%)]	10 (2.4)	6 (1.5)	.313

Abbreviations: ACE, angiotensin converting enzyme; AMI, acute myocardial infarction; BMI, body mass index; BP, blood pressure; CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; IABP, intra-aortic balloon pump; LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery; TIMI, thrombolysis in myocardial infarction.

overall worse prognosis.[31] Our results show that, despite reducing peak CK and TnT, ticagrelor had no clinical impact in terms of MACCE reduction, despite tending to be lower in ticagrelor-loaded patients. The impact of ticagrelor as a cardioprotective strategy on MACCE or other hard endpoints has been controversial. The PLATO trial showed that ticagrelor reduced death from vascular causes, stroke, and MI in ACS patients.[32] Also, in a network meta-analysis comparing the

Table 2. In-Hospital Outcomes After Propensity Score Matching.

	Clopidogrel (n = 413)	Ticagrelor (n = 413)	P Value
Intrahospital atrioventricular block [n (%)]	22 (5.3)	27 (6.6)	.451
Intrahospital pacemaker or ICD [n (%)]	14 (3.4)	14 (3.4)	.990
Medications at discharge [n (%)]			
β-blocker	381 (95.3)	328 (81.6)	<.001
ACE inhibitor	316 (79.0)	208 (51.7)	<.001
Statin	393 (98.3)	386 (96.0)	.058
LVEF at discharge [n (%)]			.202
Severe (<35%)	51 (12.3)	49 (11.9)	
Moderate (35%-45%)	98 (23.7)	99 (24.0)	
Mild (45%-55%)	84 (20.3)	108 (26.2)	
Good (>55%)	180 (43.6)	157 (38.0)	

Abbreviations: ACE, angiotensin converting enzyme; ICD, implantable cardioverter defibrillator; LVEF, left ventricle ejection fraction.

Table 3. Main Outcomes.

	Clopidogrel (n = 413)	Ticagrelor (n = 413)	P Value
Peak CK (U/L) (Q1-Q3)	1726.50 (1044.75-3425.00)	1405.50 (730.25-2491.00)	<.001[a]
Peak Troponin T (ng/mL) (Q1-Q3)	4.45 (2.56-7.88)	3.58 (1.73-6.59)	<.001[a]
MACCE [n (%)]			.129
Death	27 (6.6)	19 (4.6)	
Stroke	7 (1.7)	2 (0.5)	
Any MI	11 (2.7)	6 (1.5)	
TLR	10 (2.4)	6 (1.5)	
No event	357 (86.7)	378 (92.0)	

Abbreviations: CK, creatine kinase; MACCE, major adverse cardiac and cerebrovascular events; MI, myocardial infarction; PIA, pre-infarction angina; TLR, target leasion revascularization.
[a]Compared by Student *t* test after logarithmic transformation.

efficacy and safety of $P2Y_{12}$ inhibitors in ACS, Navarese et al showed that ticagrelor reduced mortality when compared to clopidogrel and prasugrel.[18] However, in a STEMI population, Hjortbak et al demonstrated that, as compared to clopidogrel, ticagrelor led to lower hospitalization for HF at 1-year follow-up, while failing to show a difference in MACCE incidence.[20] It is worth noting that many studies showing a reduction in mortality enrolled ACS patients, whether it referred to STEMI or not.[19,32,33] STEMI-patients present with complete coronary occlusion,[34] thus being more prone to IR injury than non-STEMI patients and the confounding factor induced by non-STEMI patients could explain the disparities between studies. Although the concentration of myocardial necrosis biomarkers has been related to final infarct size, as evaluated by cardiac magnetic resonance (CMR),[35] factors like the timing of blood sample withdrawal and the washout from the necrotic myocardium underperforms contemporary cardiac imaging modalities, which can evaluate both the area at risk and better estimate final infarct size.[31]

While ticagrelor is related to smaller infarct sizes when compared to clopidogrel,[36,37] it is important to consider that clopidogrel is a prodrug, requiring a two-step metabolic activation process that leads to a delay between when the patient is given the drug and when an adequate plasma level is reached.[38,39] This could explain why clopidogrel loading did not result in a further reduction in CK and TnT levels, as the timing of administration might not have been sufficient to allow for proper metabolism and adequate therapeutic plasma levels. This aspect is also infrequently taken into consideration in most studies comparing ticagrelor to clopidogrel. However, while this might interfere with platelet reactivity, in itself suggested to be potentially and partially cardioprotective,[20,40] the potency of $P2Y_{12}$ inhibition does not linearly translate to smaller infarct sizes.[20] In a study comparing ticagrelor to cangrelor, Ubaid et al demonstrated that, while cangrelor led to more potent $P2Y_{12}$ inhibition when compared to ticagrelor, it did not lead to further cardioprotection.[41] This adds further support to the thesis that the pleiotropic effects attributed to ticagrelor, which are not a class effect, are the main driver behind its cardioprotective properties.[20] Another important aspect to consider in our study is that ticagrelor-loaded patients presented with an overall better baseline health status, which might have influenced the results, despite the lack of difference in MACCE incidence.

Our results show that PIA reduces peak CK, but not TnT, suggesting that its cardioprotective effect occurs in the earliest MI stages.[42] In our study, patients with PIA and loaded with ticagrelor had similar enzymatic activity to patients treated with ticagrelor who did not present PIA and the lack of inter-action between each other suggests that there was no
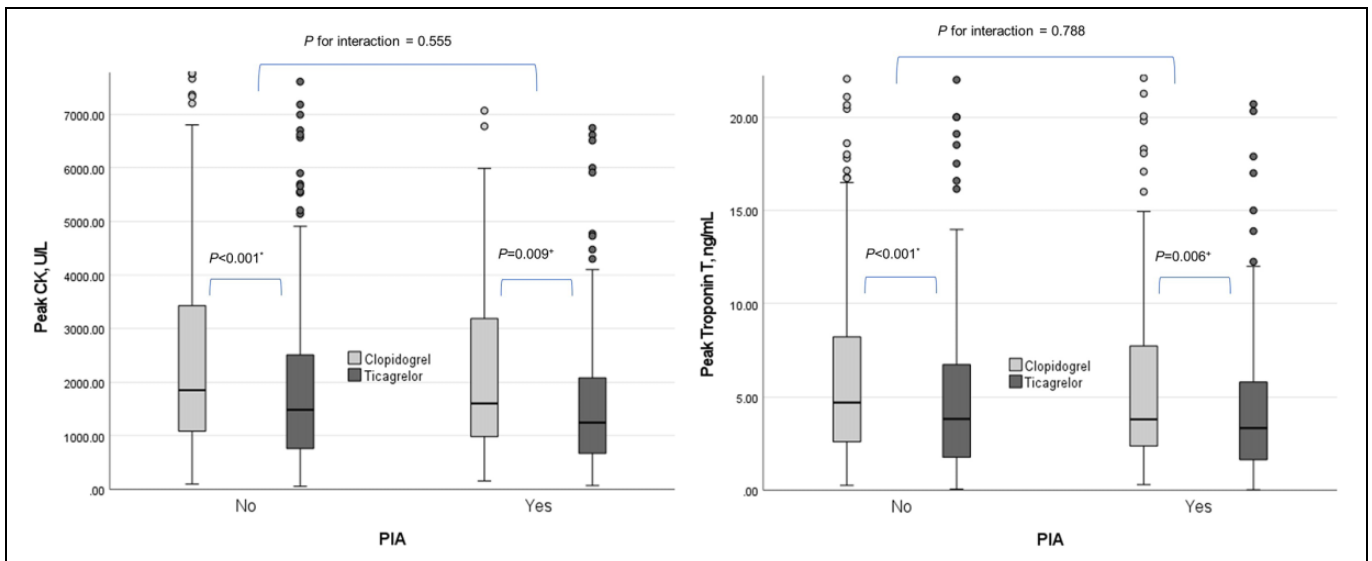
Figure 2. Distribution of peak CK and peak troponin T according to PIA status. CK indicates creatinine kinase; PIA, pre infarction angina. [+]Compared by Student t test after logarithmic transformation.
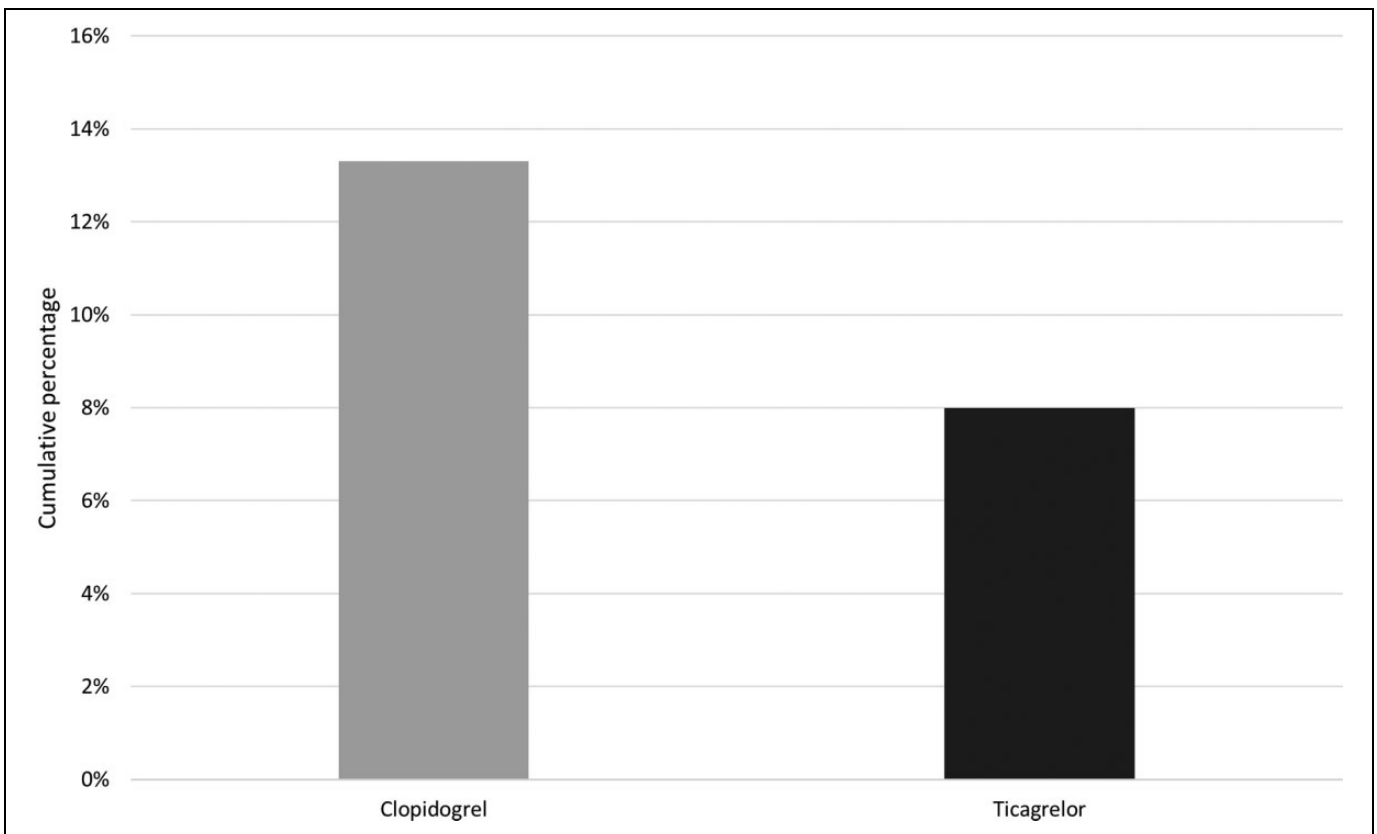

Figure 3. Proportion of cumulative major cardiac and cerebrovascular events (MACCE) according to clopidogrel or ticagrelor loading.

synergistic effect. It is not clear how PIA exerts cardioprotection, but it is thought to involve the activation of signaling pathways, among which the RISK and SAFE pathways, which ultimately target the MPTP.[3,43,44] As previously described, it is believed that ticagrelor exerts its cardioprotective effect through these pathways by enhancing local concentration of adenosine. In a preclinical trial in rabbits, Yang et al showed that when cangrelor, a $P2Y_{12}$ inhibitor, was combined with ischemic preconditioning, there was no additional cardioprotection when compared to cangrelor alone, suggesting they acted through a similar mechanism.[45] Therefore, this could explain the lack of additive effect in our study,[46] as the
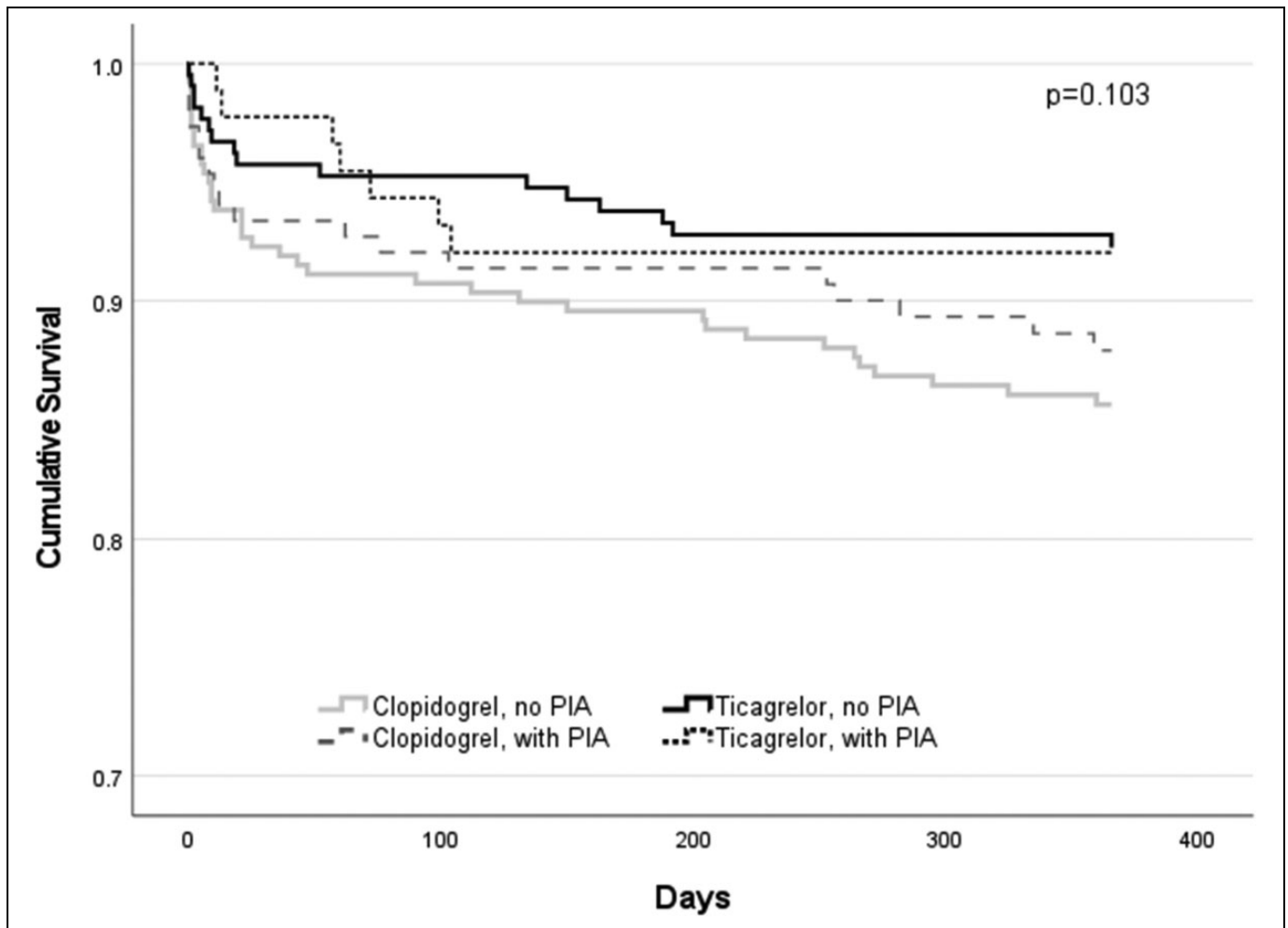
Figure 4. Kaplan-Meier curves for cumulative survival. *P* value represents the difference between groups. PIA indicates pre infarction angina.

cardioprotection provided by PIA might be superimposed by ticagrelor loading. However, there is still an advantage in ticagrelor loading, and this benefit, even if slight, is better than no benefit at all, especially among patients without PIA.

It is also possible that the current medical therapy for STEMI associated with PPCI is optimized in such a way that cardioprotection is already at its possible maximum.[47] This would possibly explain, in part, why most clinical trials testing other cardioprotective strategies show disappointing results.[48,49] In some clinical trials aimed to evaluate cardioprotective strategies, this may very well be due to a lower than anticipated event rate in an era where state-of-the-art, timely reperfusion, has already proved to be so effective that a small but nevertheless significant infarct size reduction might not have clinical impact.[47] In our series, nearly 90% of patients presented with Killip class 1-2 and a follow-up mortality rate of around 5%. Hence, a clinically meaningful benefit derived from the differences in biomarkers release would be hard to demonstrate. Finally, it is reasonable to consider that the observed benefits of ticagrelor might be more a reflection of a better STEMI-care in later years where ticagrelor replaced clopidogrel in most STEMIs than an advantage of the drug itself.

Because cardioprotection strategies aiming to mitigate the IR injury phenomenon have consistently been shown to reduce infarct sizes in a preclinical setting,[2,3,46,50] and even in some clinical trials,[51-53] a possible future direction would be to study cardioprotection maneuvers or drugs that signal different cardioprotective mechanisms in a population which benefits the most from cardioprotection on top of current STEMI-care (e.g., patients presenting with large anterior infarcts and heart failure).[47]

By sharing some of signaling cardioprotective pathways, our data suggests that ticagrelor loading might overtake the preconditioning effect afforded by PIA and might as well limit any postconditioning maneuver, which should be taken in consideration in future trials. Having those pathways already saturated after ticagrelor loading is a possible explanation. Of course, we cannot neglect the fact that its more potent antiplatelet effect might also have a role in limiting microvascular embolization and hence infarct size.[54] In fact, to take a step forward in current STEMI-care, the microvascular compartment cannot be overlooked anymore despite optimal epicardial flow restoration by PPCI.[55] Coronary microembolization is a

complex and multifactorial phenomenon caused by either device manipulation during PCI or spontaneous embolization of atherothrombotic plaque debris and clots. This leads to physical obstruction that causes micro-infarcts and to the release of soluble substances causing endothelial dysfunction, vasoconstriction and platelet aggregation. Leucocyte recruiting enhances the inflammatory response, culminating in cardiomyocyte death and contractile dysfunction.[54] Interestingly, although platelets are thought to play a key role in attaching and facilitating leucocyte infiltration into tissues, animal experiments have shown that preventing the P-selectin-dependent platelet-leucocyte complex does not improve myocardial salvage nor leucocyte transmigration.[56] Therefore, the relative potency of an antiplatelet agent is not a guarantee for a better microvascular reperfusion.

At discharge, there was no difference in LVEF between groups. This could be partly explained by our stratification in 4 groups, according to a range of LVEF values. Regarding HF, by only evaluating LVEF, we are only identifying patients with HF with reduced ejection fraction, as we did not take HF signs and symptoms into consideration for this study. Another important aspect is that we may have overlooked an impact in cardiac remodeling that is not possible to identify from the evaluation of LVEF alone. The HEALING-AMI trial showed that, despite absolute LVEF being similar across the study sample, ticagrelor was associated with positive left ventricle remodeling.[17] This could explain the lower hospitalization for HF, as described in the referred studies above, and be the main endpoint to evaluate in further studies of cardioprotection.

Limitations

There are some limitations to our study. Firstly, this is a retrospective analysis, which has its inherent limitations, and despite using PS to reduce the treatment assignment bias, it is possible that other confounders were still present. We did not evaluate hospitalization due to HF, which is an important clinical endpoint regarding the prognosis, mortality, and disease severity. We also did not take the value of NT-proBNP into consideration, as well as the variation in left ventricle diastolic or systolic volumes at 1 year follow-up, which could be better indicators of adverse cardiac remodeling.

Despite the correlation between peak CK and TnT with imaging and clinical outcomes,[57] we did not use cardiac magnetic resonance or scintigraphy to evaluate infarct size, which are more rigorous in detecting true differences in infarct size and cardioprotective effects, as well as detecting the salvaged myocardia per the area at risk.[58] We acknowledge that this may influence the validity of our results.

The interval between prodromal angina and its impact in microvascular obstruction in previous reports is wide, ranging from 48 h[59] up to 3 months[60] before STEMI. Because it is arguable that those patients who have PIA more closely to the index event may benefit the most from the preconditioning stimulus, narrowing the definition of PIA to 24 to 72 h before STEMI could have had enhanced the effect.

We also did not consider whether hypertensive patients presented with left ventricle hypertrophy, which has been suggested to interfere with cardioprotection.[61] Regarding clopidogrel loading, we did not perform genetic testing or platelet function assays. This could partly explain the lower efficiency of clopidogrel when compared to ticagrelor, as it has been shown that certain CYP isoenzymes result in a lower metabolism, leading to lower plasma clopidogrel concentrations.[38,39]

Conclusions

In this propensity score-matched study of STEMI patients, we demonstrated that ticagrelor reduced infarct size. This effect is independent of PIA, and there was no synergic effect between $P2Y_{12}$ inhibitor loading and PIA. The reduction of infarct size did not translate into better clinical outcomes, as the incidence of cumulative MACCE was not significantly different between groups. Since ticagrelor may significantly modulate infarct size as compared to clopidogrel, this should be taken into account in upcoming trials aiming to study cardioprotection in STEMI-patients.

Authors' Note

Author Contributions

All authors contributed to the study conception and design. Material preparation and data collection were performed by João Pedro Faria, André Luz, André Alexandre, David Sá Couto, Ricardo Costa, Andreia Campinas and André Frias. Data analysis was performed by João Pedro Faria, André Luz, Pedro Oliveira, Bruno Brochado, Raquel Santos, João Silveira and Severo Torres. The first draft of the manuscript was written by João Pedro Faria and André Luz and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

Funding

ORCID iDs

João Pedro Faria https://orcid.org/0000-0001-7144-6737
André Alexandre https://orcid.org/0000-0003-2465-2217

Supplemental Material

Supplemental material for this article is available online.

References

1. Ibanez B, James S, Agewall S, et al. 2017 ESC guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. *Eur Heart J*. 2018;39(2):119-177. doi:10.1093/eurheartj/ehx393

2. Heusch G. Myocardial ischaemia–reperfusion injury and cardioprotection in perspective. *Nat Rev Cardiol*. 2020;17(12):773-789. doi:10.1038/s41569-020-0403-y

3. Hausenloy DJ, Yellon DM. Ischaemic conditioning and reperfusion injury. *Nat Rev Cardiol*. 2016;13(4):193-209. doi:10.1038/nrcardio.2016.5

4. Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. *N Engl J Med*. 2007;4(B):1121-1135. doi:10.1016/S1520-765X(02)90013-1

5. Heusch G, Gersh BJ. The pathophysiology of acute myocardial infarction and strategies of protection beyond reperfusion: a continual challenge. *Eur Heart J*. 2017;38(11):774-784. doi:10.1093/eurheartj/ehw224

6. Hausenloy DJ, Botker HE, Engstrom T, et al. Targeting reperfusion injury in patients with ST-segment elevation myocardial infarction: trials and tribulations. *Eur Heart J*. 2017;38(13):935-941. doi:10.1093/eurheartj/ehw145

7. Vilahur G, Gutiérrez M, Casani L, et al. Protective effects of ticagrelor on myocardial injury after infarction. *Circulation*. 2016;134(22):1708-1719. doi:10.1161/CIRCULATIONAHA.116.024014

8. Vilahur G, Gutiérrez M, Casani L, et al. P2Y12 antagonists and cardiac repair post-myocardial infarction: global and regional heart function analysis and molecular assessments in pigs. *Cardiovasc Res*. 2018;114(14):1860-1870. doi:10.1093/cvr/cvy201

9. Birnbaum Y, Tran D, Chen H, Nylander S, Sampaio LC, Ye Y. Ticagrelor improves remodeling, reduces apoptosis, inflammation and fibrosis and increases the number of progenitor stem cells after myocardial infarction in a rat model of ischemia reperfusion. *Cell Physiol Biochem*. 2019;53(6):961-981. doi:10.33594/000000189

10. Heusch G. Critical issues for the translation of cardioprotection. *Circ Res*. 2017;120(9):1477-1486. doi:10.1161/CIRCRESAHA.117.310820

11. 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(9):872-877. doi:10.1016/j.jacc.2013.09.067

12. Cattaneo M, Schulz R, Nylander S. Adenosine-mediated effects of ticagrelor: evidence and potential clinical relevance. *J Am Coll Cardiol*. 2014;63(23):2503-2509. doi:10.1016/j.jacc.2014.03.031

13. Armstrong D, Summers C, Ewart L, Nylander S, Sidaway JE, Van Giezen JJJ. Characterization of the adenosine pharmacology of ticagrelor reveals therapeutically relevant inhibition of equilibrative nucleoside transporter 1. *J Cardiovasc Pharmacol Ther*. 2014;19(2):209-219. doi:10.1177/1074248413511693

14. Kim HK, Lim KS, Kim SS, Na J-Y. Ticagrelor does not affect left ventricular remodeling following acute myocardial ischemia. *Med Biol Sci Eng*. 2021;4(1):34-41. doi:10.30579/mbse.2021.4.1.34

15. You SC, Rho Y, Bikdeli B, et al. Association of ticagrelor vs clopidogrel with net adverse clinical events in patients with acute coronary syndrome undergoing percutaneous coronary intervention. *JAMA*. 2020;324(16):1640-1650. doi:10.1001/jama.2020.16167

16. Vercellino M, Sànchez FA, Boasi V, et al. Ticagrelor versus clopidogrel in real-world patients with ST elevation myocardial infarction: 1-year results by propensity score analysis. *BMC Cardiovasc Disord*. 2017;17(1):1-11. doi:10.1186/s12872-017-0524-3

17. Park Y, Koh JS, Lee JH, et al. Effect of ticagrelor on left ventricular remodeling in patients with ST-segment elevation myocardial infarction (HEALING-AMI). *JACC Cardiovasc Interv*. 2020;13(19):2220-2234. doi:10.1016/j.jcin.2020.08.007

18. Navarese EP, Khan SU, Kołodziejczak M, et al. Comparative efficacy and safety of oral P2Y$_{12}$ inhibitors in acute coronary syndrome: network meta-analysis of 52 816 patients from 12 randomized trials. *Circulation*. 2020;142(2):150-160. doi:10.1161/CIRCULATIONAHA.120.046786

19. Chen Q, Zhang Y, Wang Z, et al. Efficacy and safety of low dose ticagrelor in patients with acute coronary syndrome: a systematic review and meta-analysis. *Postgrad Med J*. 2020;96(1141):693-702. doi:10.1136/postgradmedj-2019-137180

20. Hjortbak MV, Olesen KKW, Seefeldt JM, et al. Translation of experimental cardioprotective capability of P2Y$_{12}$ inhibitors into clinical outcome in patients with ST-elevation myocardial infarction. *Basic Res Cardiol*. 2021;116(1):36. doi:10.1007/s00395-021-00870-y

21. Fontes Oliveira M, Costa R, Frias A, et al. Pre-infarction angina is associated with improved prognosis in diabetic patients with ST-elevation myocardial infarction-data from a contemporary cohort. *Coron Artery Dis*. 2021;32(5):375-381. doi:10.1097/MCA.0000000000000968

22. Lønborg J, Kelbæk H, Vejlstrup N, et al. Influence of pre-infarction angina, collateral flow, and pre-procedural TIMI flow on myocardial salvage index by cardiac magnetic resonance in patients with ST-segment elevation myocardial infarction. *Eur Heart J Cardiovasc Imaging*. 2012;13(5):433-443. doi:10.1093/ejechocard/jer296

23. Luz A, Santos M, Rodrigues P, et al. Preinfarction angina: clinical significance and relationship with total ischemic time in patients with ST-elevation myocardial infarction. *Coron Artery Dis*. 2015;26(1):22-29. doi:10.1097/MCA.0000000000000160

24. Kloner RA, Przyklenk K, Shook T, Cannon CP. Protection conferred by preinfarct angina is manifest in the aged heart: evidence from the TIMI 4 trial. *J Thromb Thrombolysis*. 1998;6(2):89-92. doi:10.1023/A:1008833101817

25. Heusch G. Nitroglycerin and delayed preconditioning in humans. *Circulation*. 2001;103(24):2876-2878. doi:10.1161/01.cir.103.24.2876

26. Reiter R, Henry TD, Traverse JH. Preinfarction angina reduces infarct size in ST-elevation myocardial infarction treated with

percutaneous coronary intervention. *Circ Cardiovasc Interv.* 2013;6(1):52-58. doi:10.1161/CIRCINTERVENTIONS.112. 973164

27. Masci PG, Andreini D, Francone M, et al. Prodromal angina is associated with myocardial salvage in acute ST-segment elevation myocardial infarction. *Eur Heart J Cardiovasc Imaging.* 2013;14(11):1041-1048. doi:10.1093/ehjci/jet063

28. Pryds K, Bottcher M, Sloth AD, et al. Influence of preinfarction angina and coronary collateral blood flow on the efficacy of remote ischaemic conditioning in patients with ST segment elevation myocardial infarction: post hoc subgroup analysis of a randomised controlled trial. *BMJ Open.* 2016;6(11):1-9. doi:10. 1136/bmjopen-2016-013314

29. Morrow DA, Antman EM, Charlesworth A, et al. TIMI risk score for ST-elevation myocardial infarction: a convenient, bedside, clinical score for risk assessment at presentation: an intravenous nPA for treatment of infarcting myocardium early II trial substudy. *Circulation.* 2000;102(17):2031-2037. doi:10.1161/01. CIR.102.17.2031

30. Sianos G, Morel M, Kappetein AP. The SYNTAX Score: an angiographic tool grading. *EuroIntervention.* 2005;1(2):219-227.

31. Stone GW, Selker HP, Thiele H, et al. Relationship between infarct size and outcomes following primary PCI patient-level analysis from 10 randomized trials. *J Am Coll Cardiol.* 2016; 67(14):1674-1683. doi:10.1016/j.jacc.2016.01.069

32. Wallentin L, Becker R, Budaj A, et al. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med.* 2009;361(11):687-696. doi:10.1056/NEJMoa0904327

33. Conrotto F, Bertaina M, Raposeiras-Roubin S, et al. Prasugrel or ticagrelor in patients with acute coronary syndrome and diabetes: a propensity matched substudy of RENAMI. *Eur Hear J Acute Cardiovasc Care.* 2019;8(6):536-542. doi:10.1177/ 2048872618802783

34. Vogel B, Claessen BE, Arnold SV, et al. ST-segment elevation myocardial infarction. *Nat Rev Dis Prim.* 2019;5(1):1-20. doi:10. 1038/s41572-019-0090-3

35. Reinstadler SJ, Feistritzer H-J, Klug G, et al. High-sensitivity troponin T for prediction of left ventricular function and infarct size one year following ST-elevation myocardial infarction. *Int J Cardiol.* 2016;202:188-193. doi:10.1016/j.ijcard.2015.09.001

36. Sabbah M, Nepper-Christensen L, Køber L, et al. Infarct size following loading with ticagrelor/prasugrel versus clopidogrel in ST-segment elevation myocardial infarction. *Int J Cardiol.* 2020;314:7-12. doi:10.1016/j.ijcard.2020.05.011

37. Khan JN, Greenwood JP, Nazir SA, et al. Infarct size following treatment with second-versus third-generation $P2Y_{12}$ antagonists in patients with multivessel coronary disease at ST-segment elevation myocardial infarction in the CvLPRIT study. *J Am Heart Assoc.* 2016;5(6):e003403. doi:10.1161/JAHA.116.003403

38. Cattaneo M. Response variability to clopidogrel: is tailored treatment, based on laboratory testing, the right solution? *J Thromb Haemost.* 2012;10(3):327-336. doi:10.1111/j.1538-7836.2011. 04602.x

39. Sibbing D, Aradi D, Alexopoulos D, et al. Updated expert consensus statement on platelet function and genetic testing for guiding $P2Y_{12}$ receptor inhibitor treatment in percutaneous coronary intervention. *JACC Cardiovasc Interv.* 2019;12(16):1521-1537. doi:10.1016/j.jcin.2019.03.034

40. Raphael Lieder H, Tsoumani M, Andreadou I, Schrör K, Heusch G, Kleinbongard P. Platelet-mediated transfer of cardioprotection by remote ischemic conditioning and its abrogation by aspirin but not by ticagrelor. *Cardiovasc Drugs Ther.* 2022. doi:10.1007/ s10557-022-07345-9

41. Ubaid S, Ford TJ, Berry C, et al. Cangrelor versus ticagrelor in patients treated with primary percutaneous coronary intervention: impact on platelet activity, myocardial microvascular function and infarct size: a randomized controlled trial. *Thromb Haemost.* 2019;119(7):1171-1181. doi:10.1055/s-0039-1688789

42. Jaffe AS, Babuin L, Apple FS. Biomarkers in acute cardiac disease: the present and the future. *J Am Coll Cardiol.* 2006;48(1): 1-11. doi:10.1016/j.jacc.2006.02.056

43. Hausenloy DJ, Lecour S, Yellon DM. Reperfusion injury salvage kinase and survivor activating factor enhancement prosurvival signaling pathways in ischemic postconditioning: two sides of the same coin. *Antioxidants Redox Signal.* 2011;14(5):893-907. doi: 10.1089/ars.2010.3360

44. Murphy E, Steenbergen C. Did a classic preconditioning study provide a clue to the identity of the mitochondrial permeability transition pore? *Circ Res.* 2013;113(7):852-855. doi:10.1161/ CIRCRESAHA.113.301950

45. Yang XM, Cui L, Alhammouri A, Downey JM, Cohen MV. Triple therapy greatly increases myocardial salvage during ischemia/ reperfusion in the in situ rat heart. *Cardiovasc Drugs Ther.* 2013; 27(5):403-412. doi:10.1007/s10557-013-6474-9

46. Cohen MV, Downey JM. The impact of irreproducibility and competing protection from $P2Y_{12}$ antagonists on the discovery of cardioprotective interventions. *Basic Res Cardiol.* 2017; 112(64). doi:10.1007/s00395-017-0653-y

47. Heusch G, Gersh BJ. Is cardioprotection salvageable? *Circulation.* 2020;141(6):415-417. doi:10.1161/CIRCULATIONAHA. 119.044176

48. Hausenloy DJ, Kharbanda RK, Møller UK, et al. Effect of remote ischaemic conditioning on clinical outcomes in patients with acute myocardial infarction (CONDI-2/ERIC-PPCI): a single-blind randomised controlled trial. *Lancet.* 2019;394(10207): 1415-1424. doi:10.1016/S0140-6736(19)32039-2

49. Luz A, Santos M, Magalhães R, et al. Lack of benefit of ischemic postconditioning after routine thrombus aspiration during reperfusion: immediate and midterm results. *J Cardiovasc Pharmacol Ther.* 2015;20(6):523-531. doi:10.1177/1074248415578171

50. Yang XM, Liu Y, Cui L, et al. Platelet $P2Y_{12}$ blockers confer direct postconditioning-like protection in reperfused rabbit hearts. *J Cardiovasc Pharmacol Ther.* 2013;18(3):251-262. doi:10.1177/ 1074248412467692

51. Gaspar A, Lourenço AP, Pereira MÁ, et al. Randomized controlled trial of remote ischaemic conditioning in ST-elevation myocardial infarction as adjuvant to primary angioplasty (RIC-STEMI). *Basic Res Cardiol.* 2018;113(3):14. doi:10.1007/ s00395-018-0672-3

52. Bøtker HE, Kharbanda R, Schmidt MR, et al. Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with

acute myocardial infarction: a randomised trial. *Lancet.* 2010; 375(9716):727-734. doi:10.1016/S0140-6736(09)62001-8

53. Heusch G. 25 Years of remote ischemic conditioning: from laboratory curiosity to clinical outcome. *Basic Res Cardiol.* 2018;113(3):15. doi:10.1007/s00395-018-0673-2

54. Kleinbongard P, Heusch G. A fresh look at coronary microembolization. *Nat Rev Cardiol.* 2022;19(4):265-280. doi:10.1038/s41569-021-00632-2

55. Niccoli G, Montone RA, Ibanez B, et al. Optimized treatment of ST-elevation myocardial infarction the unmet need to target coronary microvascular obstruction as primary treatment goal to further improve prognosis. *Circ Res.* 2019;125(2):245-258. doi:10.1161/CIRCRESAHA.119.315344

56. Starz C, Härdtner C, Mauler M, et al. Elevated platelet–leukocyte complexes are associated with, but dispensable for myocardial ischemia–reperfusion injury. *Basic Res Cardiol.* 2022;117(1):61. doi:10.1007/s00395-022-00970-3

57. Chia S, Senatore F, Raffel OC, Lee H, Wackers FJT, Jang IK. Utility of cardiac biomarkers in predicting infarct size, left ventricular function, and clinical outcome after primary percutaneous coronary intervention for ST-segment elevation myocardial infarction. *JACC Cardiovasc Interv.* 2008;1(4):415-423. doi:10.1016/j.jcin.2008.04.010

58. Bøtker HE, Hausenloy D, Andreadou I, et al. Practical guidelines for rigor and reproducibility in preclinical and clinical studies on cardioprotection. *Basic Res Cardiol.* 2018;113(5):1-73. doi:10.1007/s00395-018-0696-8

59. Niccoli G, Scalone G, Cosentino N, et al. Protective effect of pre-infarction angina on microvascular obstruction after primary percutaneous coronary intervention is blunted in humans by cardiovascular risk factors. *Circ J.* 2014;78(8):1935-1941. doi:10.1253/circj.CJ-14-0194

60. Lønborg J, Vejlstrup N, Kelbæk H, et al. Exenatide reduces reperfusion injury in patients with ST-segment elevation myocardial infarction. *Eur Heart J.* 2012;33(12):1491-1499. doi:10.1093/eurheartj/ehr309

61. Kleinbongard P, Bøtker HE, Ovize M, Hausenloy DJ, Heusch G. Co-morbidities and co-medications as confounders of cardioprotection—does it matter in the clinical setting? *Br J Pharmacol.* 2020;177(23):5252-5269. doi:10.1111/bph.14839