

Pharmacogenomic Approach to Selecting Antiplatelet Therapy in Acute Coronary Syndromes: PHARMCLO trial

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Pharmacogenomic Approach to Selecting Antiplatelet Therapy in Acute Coronary Syndromes: PHARMCLO trial

Short title: PHARMCLO: a prospective, randomized, multicenter study

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ABSTRACT

Background: Clopidogrel is still frequently used in patients with acute coronary syndromes (ACS), but its efficacy is hampered by interpatient response variability, due to genetic polymorphisms associated with clopidogrel metabolism.

Objective: To evaluate whether selecting antiplatelet therapy (clopidogrel, prasugrel or ticagrelor) on the basis of a patient's genetic and clinical characteristics leads to better clinical outcomes in comparison with the standard of care, which bases the selection on clinical characteristics alone

Methods: Patients hospitalised for ACS were randomly assigned to standard of care or pharmacogenomic arm, which included the genotyping of ABCB1, CYP2C19*2, CYP2C19*17 using an ST Q3 system that provide the data within 70 minutes at each patient's bedside. The patients were followed up for 12 ± 1 months for the primary composite endpoint of cardiovascular death and the first occurrence of non-fatal myocardial infarction, non-fatal stroke and BARC 3 to 5-defined major bleeding.

Results: After enrolling 888 patients, the study was prematurely stopped. Clopidogrel was used more frequently in the standard of care arm (50.7% vs 43.3%), ticagrelor in the pharmacogenomic arm (42.6% vs 32.7%; $P=0.02$), and prasugrel was equally used in both arms. The primary endpoint occurred in 71 patients (15.9%) in the pharmacogenomic arm and in 114 (25.9%) in the standard of care arm (hazard ratio 0.58; 95% confidence interval [CI] 0.43 to 0.78; $P<0.001$).

Conclusion: A personalised approach to selecting antiplatelet therapy for ACS patients may reduce ischemic and bleeding events (PHARMCLO - NCT03347435).

Clinical Trial: www.clinicaltrial.gov NCT03347435

Keywords: Pharmacogenomic, clopidogrel, acute coronary syndromes

Condensed Abstract: Clopidogrel is still frequently used in patients with acute coronary syndromes, but its efficacy is affected by interpatient response variability, due to genetic polymorphisms. This study of 888 patients combined genetic and clinical variables to personalise the choice of P2Y₁₂ receptor antagonist. The primary endpoint of ischemic and bleeding events occurred in the 15.9% of the patients in the pharmacogenomic arm, and 25.9% of those in the standard of care arm (hazard ratio 0.58; 95% confidence interval [CI] 0.43 to 0.78; $P<0.001$). A personalised approach to the selection of antiplatelet therapy may improve clinical outcomes in ACS.

Abbreviations

1. ACS = Acute Coronary Syndromes
2. DNA = Deoxyribo Nucleic Acid
3. IVD = In Vitro Diagnosis
4. PCR = Polymerase Chain Reaction
5. BMI: body mass index
6. PCI: percutaneous coronary intervention
7. CABG: coronary artery by-pass grafting
8. COPD: chronic obstructive pulmonary disease
9. NSTEMI: non-ST-elevation myocardial infarction
10. ACEi: angiotensin-converting enzyme inhibitors
11. ARB: angiotensin receptor blockers

Introduction

Dual antiplatelet therapy with aspirin and a P2Y₁₂ receptor antagonist is the mainstay of the treatment of acute coronary syndromes. Prasugrel and ticagrelor have proved to be superior to clopidogrel in preventing ischemic events, but lead to an increased risk of bleeding complications. The International guidelines (1-4) strongly suggest using front-line treatment with prasugrel or ticagrelor, and only using clopidogrel if they are unavailable or contraindicated. However, the use of more potent antiplatelet drugs involves a fundamental trade-off between decreasing the risk of ischemia and increasing the risk of bleeding. For this reason, clopidogrel is frequently chosen in the real world (5-8) but, when balancing ischemic/bleeding risks, only the clinical characteristics of individual patients are taken into account.

Clopidogrel leads to substantial variability in patient responses and this limits its effectiveness (9). In addition to clinical factors, the reasons for this variability include genotypes: loss or gain-of-function polymorphisms in the cytochrome P450 2C19 gene are frequently observed and are not only responsible for increasing/reducing enzymatic activity, but also for inducing high/low on-treatment platelet reactivity after clopidogrel exposure, both of which affect its effectiveness in preventing ischemic and bleeding events (10-13). Genetic variants of the genes regulating clopidogrel absorption, such as ABCB1, have also been shown to affect clopidogrel bioavailability, on-treatment platelet reactivity and clinical outcomes (14).

It can therefore be hypothesised that knowing a patient's genetic profile of clopidogrel metabolism may lead to more personalised, and therefore more efficient antiplatelet therapy. This study was designed to test the hypothesis that selecting antiplatelet therapy on the basis of a combination of genetic information and a patient's clinical characteristics would lead to better clinical outcomes in comparison with the standard of care based on clinical characteristics alone.

Methods

Patient population

Unselected patients of European ancestry who were hospitalised because of ST-segment elevation or non-ST-segment elevation acute coronary syndromes were considered eligible for enrolment. The diagnosis of acute coronary syndromes was based on the presence of at least two of the following criteria: 1) ischemic symptoms at rest lasting >20 minutes; 2) electrocardiographic changes with ST-segment elevation or depression of at least 1 mm in two contiguous leads; and 3) the typical rise and fall of cardiac biomarker troponin I or T levels above the 99th percentile of the upper reference limit.

The major exclusion criteria were: 1) an inability to provide informed consent or follow study procedures; 2) any contraindication to the use of P2Y₁₂ receptor antagonists; 3) a life expectancy of <1 year; 4) thrombolytic therapy within the previous 24 hours; 5) enrolment in another randomized trial or observational registry; and 6) prior knowledge of the patients' ABCB1, CYP2C19*2 or CYP2C19*17 genotype.

The study protocol was approved by the Ethics Committee of the Coordinating Centre, and afterwards by the Ethics Committee of each participating centre; all of the patients gave their written informed consent before taking part in the trial.

Randomization

Immediately after the diagnosis of acute coronary syndrome, the patients were randomly assigned to the strategy of selecting a P2Y₁₂ receptor antagonist on the basis of their clinical characteristics plus the genotyping results, or on the basis of their clinical characteristics alone. An automatic telephone randomisation system was used to assign the patients to treatment in a 1:1 ratio based on a centralised list. The randomization list was stratified by diagnosis (ST-

segment elevation vs all the rest) and by centre using the SAS PLAN procedure (SAS Institute, Cary, North Carolina). The investigators had to digitise the patient's date of birth, diagnosis and site number by means of an interactive voice system in order to obtain the assigned allocation. The data were confirmed by e-mail, and the print-out was included in the patient's case report form.

Genotyping

The patients assigned to the strategy that included genotyping underwent genetic testing for ABCB1 3435 (7q21.1; rs1045642), CYP2C19*2 (10q24.1-q24.3; rs4244285) and CYP2C19*17 (10q24.1-q24.3; rs12248560). Blood samples were taken from all of the patients randomized to the pharmacogenomic arm for DNA extraction. The genotyping was carried out using the ST Q3 system, a compact platform enabling the laboratory analysis of DNA by means of real-time PCR. The ST Q3 is designed as a low entry-cost portable system for foolproof use by unskilled personnel as a point-of-care instrument. The reaction takes place in a disposable lab-on-chip, which is pre-functionalized with all of the required reagents. The user only has to add the sample by means of a simple loading system, after which the disposable device self-seals and provides the results in approximately 70 minutes (15) (Appendix B- Supplementary Methods).

Study procedure and follow-up

The patients randomized to the arm that included genotyping received one of the P2Y₁₂ receptor antagonists (clopidogrel, prasugrel or, ticagrelor) on the basis of an algorithm that considered the results of genetic testing in addition to clinical variables. Patients already taking P2Y₁₂ antagonists could be enrolled but, if switching was necessary in order to implement the

protocol, it was not counted as switching. Switching was counted only if it occurred after the selection of the definite P2Y₁₂ receptor antagonist.”

It is important to underline that the genetic algorithm was designed to consider the three genes simultaneously, but the ultimate decision concerning antiplatelet therapy also took into account the patient’s clinical characteristics and was left to the discretion of the prescribers.

(Appendix B- Supplementary Methods, Online Figure 1).

The clinical variables used to individualize the choice of P2Y₁₂ receptor antagonist were age, weight, ischemic risk, bleeding risk, diabetes, prior history of stroke/TIA or intracranial bleeding, history of bleeding, active bleeding, anemia, chronic kidney disease.

Given the TRITON-TIMI-38 trial findings and the exclusion criteria, prasugrel was not prescribed for: 1) patients whose coronary anatomy was unknown; 2) those at high bleeding risk (particularly those with a history of stroke or TIA, those aged >75 years, and those with a body weight of <60 kg); 3) those receiving fibrinolytic therapy within the previous 24 hours; 4) those with active internal bleeding; 5) those with severe liver disease; 6) those taking oral anticoagulant therapy that could not be stopped; or 7) those with known clinically important thrombocytopenia (16).

Given the PLATO trial exclusion criteria, ticagrelor was contraindicated in: 1) patients with active pathological bleeding; 2) those with a history of intracranial bleeding; 3) those requiring dialysis, 4) those taking oral anticoagulant therapy that could not be stopped; 5) those with known clinically important thrombocytopenia; 6) those receiving fibrinolytic therapy within the previous 24 hours; and 7) those taking concomitant therapy with strong CYP3A inhibitors or inducers (17).

In the standard of care arm, the decision on to which P2Y₁₂ receptor antagonist to use was based on clinical characteristics as described above and the clinicians' preference. The patients were followed up for 12 months by means of outpatient visits or standardized telephone contacts scheduled 1, 6 and 12 $\pm$ 1 months after randomization.

Study outcomes

The primary endpoint was the composite of cardiovascular death and the first occurrence of non-fatal myocardial infarction, non-fatal stroke and BARC 3 to 5-defined major bleeding within 12 months of randomization.

The secondary end-point was the composite of the primary endpoint plus the occurrence of definite or probable stent thrombosis. Secondary non-pre-specified endpoints were the composite of the first occurrence of the ischemic endpoints (cardiovascular death, non-fatal myocardial infarction and non-fatal stroke) and the composite of the first occurrence of the BARC 3 to 5-defined major bleeding (Appendix C- Follow-up and endpoint definitions).

All of the events were adjudicated by an independent Clinical Events Committee consisting of three cardiologists who were unaware of the study group assignments. Two cardiologists reviewed the medical records and, in the case of disagreement, the opinion of the third member was required. Only adjudicated endpoints were considered for the statistical analysis.

Statistical analysis

The sample size was calculated on the assumption that the cumulative incidence of the primary endpoint in the standard of care arm after 12 months would be 25%. Given a relative risk reduction in the pharmacogenomic arm of 20%, 95% power and a type alpha error of 5%, the calculated sample size was 1,806 patients in each arm.

Descriptive statistics were used to compare the baseline characteristics of the two groups in order to test the randomisation process. The primary analysis was based on the intention-to-treat principle. Cox proportional hazard models were used to analyze the data relating to the primary and secondary endpoints. The proportional hazards assumption for the Cox regression model was confirmed using the Schoenfeld residuals test. An Andersen–Gill intensity model analysis was not pre-specified, but was carried out in order to account for repeated occurrences of all of the components of the primary endpoint during the study period, using a time-dependent model.

The cumulative incidence of the endpoints during the 12 months of follow-up was graphically represented by means of Aalen-Johansen curves, and the significance of the differences between the sub-distribution of the hazards was tested using the Fine-Gray model. All of the tests were two-sided at a significance level of 0.05. No interim analysis was performed and no multiplicity test correction was made. The statistical analyses were made using the R-package Survival (version 2.41-3) and R Statistical Software version 3.1.3.

Results

A total of 888 patients were recruited between 12 June 2013 and 18 February 2015; 448 patients were randomized to the pharmacogenomic arm and 440 to the standard of care arm. This represents 24.6% of the pre-specified sample size because, on 18 February 2015, the Ethics Committee of Modena (Italy) required that the trial should be prematurely stopped and all of the patients followed up as planned because of the lack of *in vitro* diagnosis (IVD) certification for the ST Q3 instrument.

The mean age of the population was 70.4 ± 12 years (range 35-97), with 28.4% of the patients aged >80 years. Virtually all of the patients (98.1%) experienced the typical rise and fall

of cardiac markers indicating an acute myocardial infarction and 96.3% underwent coronary angiography. Seventy-three percent underwent revascularization: 62.4% by means of a percutaneous coronary intervention and 11% by means of coronary artery by-pass grafting. All of these characteristics indicate a high-risk study population. The concomitant treatments were those currently used for patients with acute coronary syndromes. The two groups were well balanced in terms of the patients' baseline characteristics (**Table 1**).

Genotypes

Table 2 shows the genotype distributions of the patients in the pharmacogenomic arm. Briefly, genotyping revealed that 47.1% had at least one copy of the ABCB1 3435 allele, and 26.4% were homozygous; 29.2% had at least one copy of the loss-of-function CYP2C19*2 allele, and 4.3% were homozygous; and 31.3% had at least one copy of the gain-of-function CYP2C19*17 allele, and 7.8% were homozygous.

The distribution of the P2Y₁₂ receptor antagonists used during the acute phase and the 12 months of follow-up in the pharmacogenomic arm was clopidogrel in 43.3% of the patients, prasugrel in 7.6%, and ticagrelor in 42.6%. The corresponding figures in the standard of care arm were clopidogrel 50.7%, prasugrel 8.4%, and ticagrelor 32.7%. The difference in the distribution of the treatments with different P2Y₁₂ receptor antagonists between the two arms was statistically significant ($P=0.02$). After the selection of the P2Y₁₂ receptor antagonist, 6.8% of the patients in the pharmacogenomic arm and 5.6% in the standard of care arm changed their P2Y₁₂ receptor antagonist at least once. Upon discharge, 6.5% of the patients in the pharmacogenomic arm and 8.2% of those in the standard of care arm were not receiving any P2Y₁₂ receptor antagonist, mainly because of misdiagnosis, normal coronary angiograms or the concomitant use of oral anticoagulants.

Outcomes

One patient in the pharmacogenomic arm was lost to follow-up. The primary endpoint occurred in 71 patients (15.9%) in the pharmacogenomic arm and 114 (25.9%) in the standard of care arm (hazard ratio 0.58; 95% confidence interval, 0.43 to 0.78; $P < 0.001$) (Central Illustration, Table 3). In patients receiving clopidogrel, the primary endpoint occurred in 48 patients in the pharmacogenomic arm (24.7%) and 79 (35.4%) in the standard of care arm (hazard ratio 0.68; 95% confidence interval 0.47-0.97; $P=0.03$) (**Figure 1**).

Definite or probable stent thrombosis was observed in only eight patients (three in the pharmacogenomic arm and five in the standard of care arm), thus precluding an outcome analysis of this secondary endpoint.

Ischemic endpoints occurred in 58 patients (13%) in the pharmacogenomic arm and 94 (21.4%) in the standard of care arm (hazard ratio 0.57; 95% confidence interval, 0.41 to 0.8; $P < 0.001$) (**Figure 2**). Bleeding endpoints occurred in 19 patients (4.2%) in the pharmacogenomic arm and 30 (6.8%) in the standard of care arm (hazard ratio 0.62; 95% confidence interval, 0.35 to 1.1; $P = 0.1$) (**Figure 3**).

Repeated ischemic and bleeding endpoints were also less frequent in the patients randomized to the pharmacogenomic arm: 85 vs 136 endpoints (hazard ratio 0.61; 95% confidence interval, 0.4 to 0.8; $P<0.001$).

Discussion

Over the last 10 years, there have been substantial advances in our understanding of the genetic variability associated with patient responses to clopidogrel treatment (10-14). However, although these genetic associations have been widely replicated and the effect sizes are

sufficiently large to be predictive in the clinical setting, there are limited examples of the use of pharmacogenetic data concerning clopidogrel metabolism to guide clinical practice (19-23).

This prospective, randomized multicenter study provides evidence that the use of genomic medicine to select P2Y₁₂ receptor antagonists can be successfully incorporated into the clinical care of patients with acute coronary syndromes. This may be considered one of the most challenging clinical settings in which to use pharmacogenetic data to guide clinical practice because of the urgency of the situation and the need to start drug treatment promptly. In our case, this was made feasible by the development of a bedside instrument capable of providing genotype results within 70 minutes of blood sampling.

In patients with acute coronary syndromes, the selection of a P2Y₁₂ receptor antagonist (clopidogrel, ticagrelor or prasugrel) is based on their individual clinical characteristics in order to obtain the best trade-off between ischemic events and bleeding complications. The main finding of the present study is that selecting treatment on the basis of genetic data related to clopidogrel metabolism in addition to considerations concerning the patients' clinical characteristics may lead to a significantly lower rate of ischemic and bleeding events in comparison with usual practice.

While it is relatively easy to explain why knowledge of a patient's genetic data may lead to a better clinical outcome, it is more difficult to explain the magnitude of this effect. Given the premature discontinuation of the study after the enrolment of only 25% of the planned number of patients it is possible that the observed differences between the two arms may simply be attributable to a chance. Alternatively, it is possible that the effect size in our sample size calculation may have been simply underestimated: a retrospective analysis of the TRITON-TIMI-38 trial by Mega et al. showed that the absolute difference in cardiovascular death, non-

fatal myocardial infarction or non-fatal stroke was 7.3% between the most and least favourable ABCB1 and 2C19*2 genetic profile, with a relative difference of 50% (14). Another possible explanation may be related to the percentage of patients receiving clopidogrel or ticagrelor in the standard of care arm. As ticagrelor proved to be more effective than clopidogrel in the PLATO trial (17), it is possible that some of the benefit observed in our study may have been related to the more frequent use of clopidogrel in the standard of care arm. Finally, the ascertainment bias associated with the single-blind nature of the study may also partially account for the magnitude of the effect size.

Genotype-guided antiplatelet therapy may be seen as an alternative approach to personalised treatment in acute coronary syndromes. Other strategies based on platelet function tests have been evaluated but all have failed to demonstrate any significant clinical benefit (24-26), and it is only recently that the de-escalation of antiplatelet therapy has been shown to be not inferior to prasugrel when based on platelet function tests (27). The major limitation of platelet function tests is the great variability of the results, but this can be overcome by genetic testing (28). Two randomized trials of personalised antiplatelet therapy based on the genotyping of Chinese patients undergoing percutaneous coronary interventions have shown a significant reduction in major adverse cardiovascular events (29,30), and our own findings randomized are in line with this although, given the premature discontinuation of the study, no definite conclusion can be drawn. A number of randomized clinical trials with structured and standardised study protocols based on CYP2C19 genotyping are ongoing, and will soon provide important results that will allow finer tuning in this field (31).randomized

Conclusions

The findings of this study demonstrate that the implementation of multiple genotyping to guide antiplatelet therapy in patients with acute coronary syndromes is feasible across different institutions. Our data also suggest that a more personalised approach to the selection of antiplatelet therapy may lead to a clinically meaningful reduction in ischemic and bleeding complications. Future studies of genotype-guided antiplatelet therapy are required to confirm these data and clarify the cost-efficacy of genotyping in the challenging setting of acute coronary syndromes before implementing it in everyday clinical practice.

CLINICAL PERSPECTIVES

Competency in medical knowledge: Dual antiplatelet therapy with aspirin and a P2Y₁₂ receptor antagonist is the mainstay of the pharmacological treatment of patients with acute coronary syndromes. The tailored selection of P2Y₁₂ receptor antagonists on the basis of genotype information concerning clopidogrel metabolism in addition to clinical variables may improve clinical outcomes.

Translational outlook: Further studies are needed to confirm these preliminary data concerning the genotyping approach to the selection of P2Y₁₂ receptor antagonists for patients with acute coronary syndrome before it can be implemented in everyday clinical practice.

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Figure Legends

Central Illustration: Cumulative incidence of the primary composite endpoint after 12 months by study arm. Aalen-Johansen curves of the cumulative incidence of the primary endpoint (cardiovascular death, non-fatal MI, non-fatal stroke and BARC 3 to 5-defined major bleeding). The differences between the sub-distribution hazards in the two study arms shown in all of the panels were tested using the Fine-Gray model. The insets show the same data on an enlarged y axis.

Figure 1: Cumulative incidence of the primary endpoint in the group of patients receiving clopidogrel. Aalen-Johansen curves of the cumulative incidence of the primary endpoint in the subgroup of patients who received clopidogrel during the study. The insets show the same data on an enlarged y axis.

Figure 2: Cumulative incidence of the ischemic endpoints after 12 months by study arm. Aalen-Johansen curves of the cumulative incidence of the ischemic endpoints. The insets show the same data on an enlarged y axis.

Figure 3: Cumulative incidence of the bleeding endpoints after 12 months by study arm. Aalen-Johansen curves of the cumulative incidence of the bleeding endpoints. The insets show the same data on an enlarged y axis.

Table 1. Demographic, clinical, angiographic and treatment characteristics of the study population.

Characteristics	All patients (No=888)	Pharmacogenomic arm (No=448)	Standard of care arm (No=440)
Demographics			
Mean age $\pm$ SD, years	70.9 ($\pm$ 12.2)	71.1 ($\pm$ 12.3)	70.7 ($\pm$ 12.1)
-<70 years	361/888 (40.6)	186/448 (41.5)	175/440 (39.8)
-70-80 years	275/888 (31.0)	130/448 (29.0)	145/440 (33.0)
->80 years	252/888 (28.4)	132/448 (29.5)	120/440 (27.2)
Female sex - No.(%)	283/888 (32.0)	153/448 (34.2)	130/440 (29.6)
Cardiovascular risk factors – No. (%)			
-Family history	198/888 (22.3)	96/448 (21.4)	102/440 (23.2)
-Hypertension	660/888 (74.3)	331/448 (73.9)	329/440 (74.8)
-Dyslipidemia	483/888 (54.4)	251/448 (56.0)	232/440 (52.7)
-Diabetes mellitus	235/888 (26.5)	113/448 (25.2)	122/440 (27.7)
-Habitual smoking	200/888 (22.5)	92/448 (20.5)	108/440 (24.6)
-BMI – No.(%)			
<25 kg/m ²	117/888 (13.2)	62/448 (13.8)	55/440 (12.5)
25-30 kg/m ²	619/888 (69.7)	311/448 (69.4)	308/440 (70.0)
>30 kg/m ²	152/888 (17.1)	75/448 (16.8)	77/440 (17.5)

History – No. (%)			
-Previous MI	191/888 (21.5)	96/448 (21.4)	95/440 (21.6)
-Previous stable angina	50/888 (5.6)	25/448 (5.6)	25/440 (5.7)
-Previous PCI ¶	169/888 (19.0)	81/448 (18.1)	88/440 (20.0)
-Previous CABG	80/888 (9.0)	43/448 (9.6)	37/440 (8.4)
-Previous stroke	63/888 (7.1)	35/448 (7.8)	28/440 (6.4)
-Peripheral arterial disease	85/888 (9.6)	40/448 (8.9)	45/440 (10.2)
-Permanent atrial fibrillation	41/888 (4.7)	22/448 (4.9)	19/440 (4.3)
-Chronic kidney disease	76/888 (8.6)	35/448 (7.8)	41/440 (9.3)
-COPD	71/888 (8.0)	33/448 (7.4)	38/440 (8.6)
Acute coronary syndrome – No. (%)			
-STEMI	244/888 (27.5)	114/448 (25.5)	130/440 (29.5)
-NSTEMI	602/888 (67.8)	316/448 (70.5)	286/440 (65)
-Unstable angina	17/888 (1.9)	7/448 (1.6)	10/440 (2.3)
-No acute coronary syndrome †	25/888 (2.8)	11/448 (2.4)	14/440 (3.2)
Coronary angiography – No. (%)			
-Angiography performed	855/888 (96.3)	433/448 (96.6)	422/440 (95.9)
-Single-vessel disease	265/855 (30.9)	124/433 (28.6)	141/422 (33.4)
-Two-vessel disease	234/855 (27.3)	125/433 (28.8)	109/422 (25.8)
-Three-vessel disease	231/855 (27.0)	119/433 (27.4)	112/422 (26.5)
-Left main coronary artery	105/855 (12.2)	60/433 (13.8)	45/422 (10.6)
-Left anterior descending coronary artery	460/855 (53.8)	236/433 (54.5)	224/422 (53.1)

-Circumflex coronary artery	315/855 (36.8)	174/433 (40.1)	140/422 (33.1)
-Right coronary artery	406/855 (47.4)	203/433 (46.8)	203/422 (48.1)
-Other vessels	265/855 (30.9)	125/433 (28.8)	140/422 (33.1)
Revascularisation – No. (%)			
PCI	532/855 (62.2)	268/433 (61.8)	264/422 (62.6)
CABG	92/855 (10.7)	49/433 (11.3)	43/422 (10.1)
Medical treatment – No. (%) ‡			
Aspirin	860/888 (97.0)	437/448 (97.6)	423/440 (96.1)
Beta-blocker	751/888 (84.6)	382/448 (85.3)	369/440 (83.9)
ACEi and ARB	658/888 (74.1)	342/448 (76.3)	316/440 (71.8)
Lipid-lowering drug	761/888 (85.6)	386/448 (86.2)	375/440 (85.2)
Calcium-channel inhibitor	243/888 (27.4)	120/448 (26.8)	123/440 (28.0)
Warfarin	48/888 (5.4)	21/448 (4.7)	27/440 (6.1)

P>0.05 for all comparisons. BMI: body mass index; MI: myocardial infarction; PCI: percutaneous coronary intervention; CABG: coronary artery by-pass grafting; COPD: chronic obstructive pulmonary disease. STEMI: ST-elevation myocardial infarction. NSTEMI: non-ST-elevation myocardial infarction. ACEi: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker.

¶ A total of 93% of the PCIs involved stenting.

† No acute coronary syndrome: this category includes patients with a misdiagnosis of acute coronary syndrome.

‡ All of the medical treatments were prescribed during hospitalisation and upon discharge.

Table 2. Frequency distribution of genetic variants and selected P2Y12 receptor antagonist

Characteristics	Pharmacogenomic arm (No=448)	Standard of care arm (No=440)
ABCB1 3435 genotype, No (%)		
Wild type (C/C)	119/448 (26.5)	NA
Heterozygous (C/T)	211/448 (47.1)	NA
Homozygous (T/T)	118/448 (26.4)	NA
CYP2C19*2 genotype, No (%)		
Wild type (*1/*1)	298/448 (66.5)	NA
Heterozygous (*1/*2)	131/448 (29.2)	NA
Homozygous (*2/*2)	19/448 (4.3)	NA
CYP2C19*17 genotype, No (%)		
Wild type (*1/*1)	273/448 (60.9)	NA
Heterozygous (*1/*17)	140/448 (31.3)	NA

Homozygous (*17/*17)	35/448 (7.8)	NA
P2Y12 receptor antagonist, No (%)*		
Clopidogrel	194/448 (43.3)	223/440 (50.7)
Prasugrel	34/448 (7.6)	37/440 (8.4)
Ticagrelor	191/448 (42.6)	144/440 (32.7)
P2Y12 receptor antagonist switch ¶	27/397 (6.8)	22/393 (5.6)
No P2Y12 receptor antagonist §	29/448 (6.5)	36/440 (8.2)

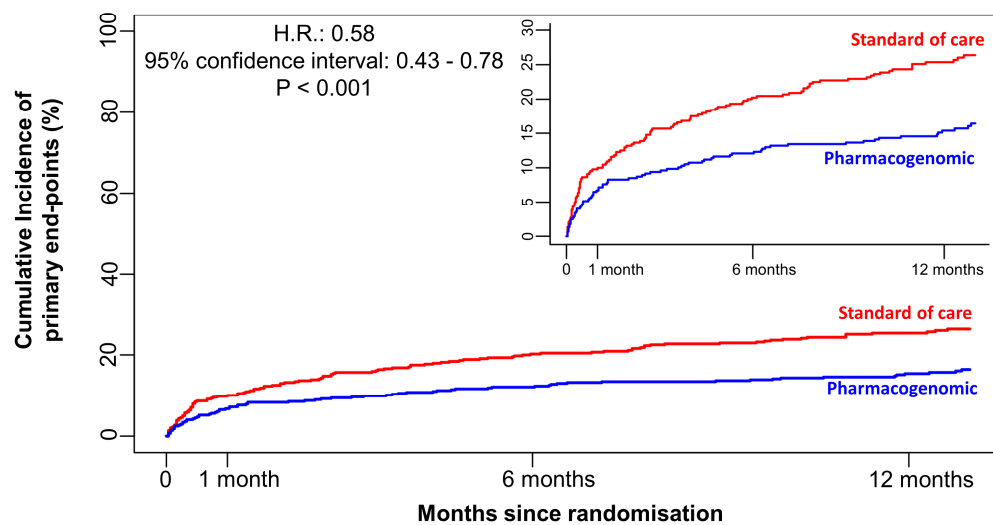
* P2Y12 receptor antagonist: P2Y12 receptor antagonist prescribed during hospitalisation and upon discharge. P value for global comparison = 0.02

§ No P2Y12 receptor antagonist: patients who did not receive any P2Y12 receptor antagonists during hospitalisation or upon discharge (because of misdiagnosis, a high bleeding risk, or concomitant oral anticoagulant therapy). ¶ P2Y12 receptor antagonist switch: any switch from one P2Y12 receptor antagonist to another during the 12-month follow-up.

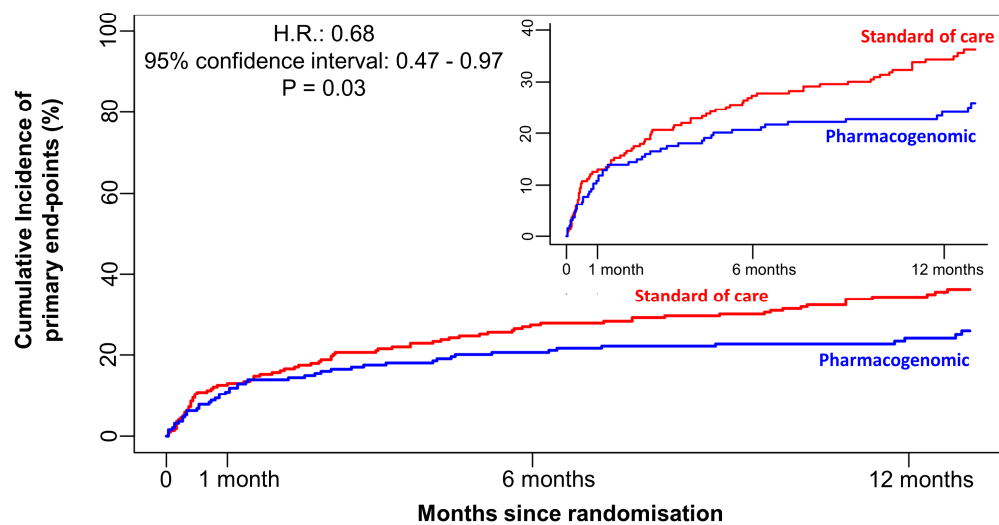
Table 3. First occurrence of the individual components of the primary composite endpoint.

Individual components of the primary composite endpoint	Pharmacogenomic arm (No=448)	Standard of care arm (No=440)	Hazard ratio (95% CI)
Cardiovascular death	28	34	
Non-fatal myocardial infarction	21	47	
-Post-PCI myocardial infarction	1	7	
-Post-CABG myocardial infarction	1	9	
Non-fatal stroke	5	7	
Combined major bleeding (BARC*3+4+5)	17	26	
Primary composite endpoint	71	114	0.58 [0.43-0.78]

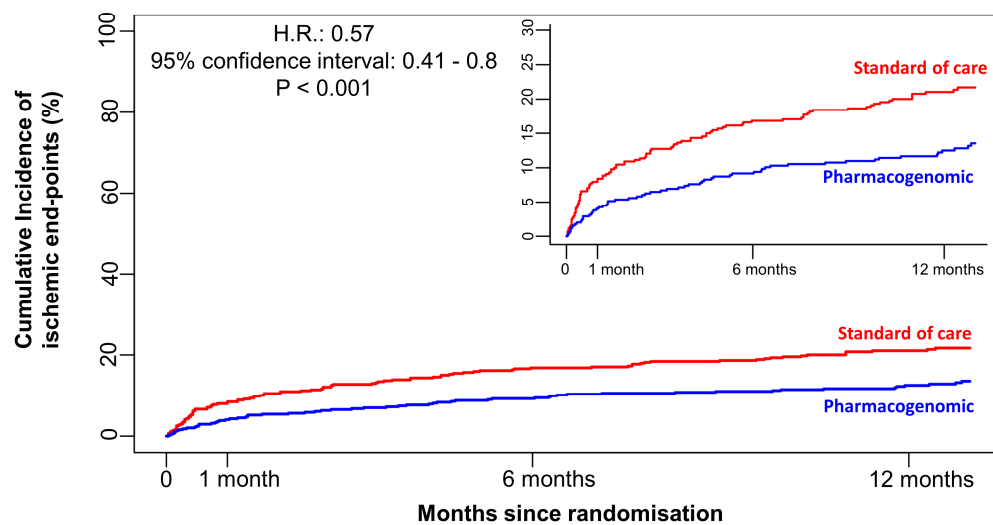
*Major bleeding according to the Bleeding Academic Research Consortium (BARC) definition

**No. at risk**

Pharmacogenomic arm	448	416	390	295
Standard of care arm	440	397	349	280

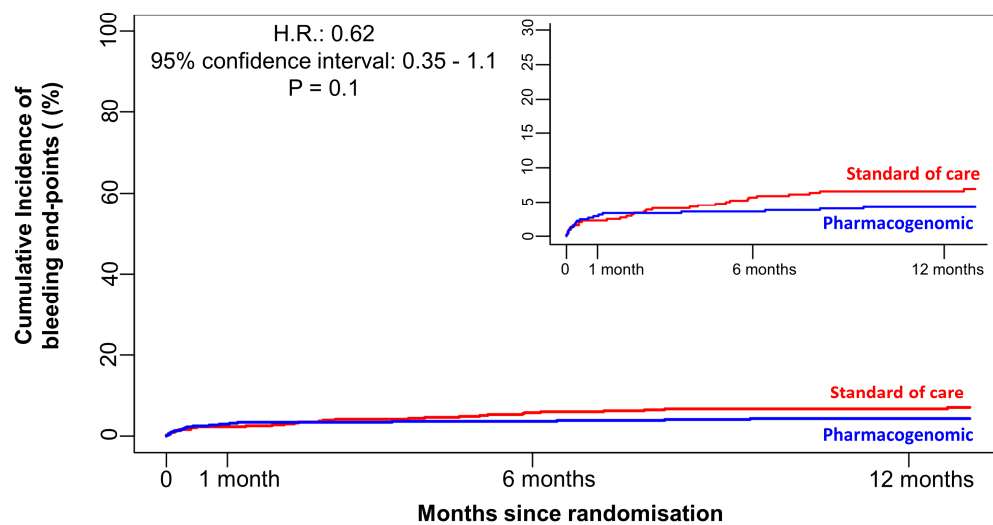
**No. at risk**

Pharmacogenomic arm	194	173	152	102
Standard of care arm	223	195	161	127



No. at risk

Pharmacogenomic arm	448	428	402	304
Standard of care arm	440	404	362	294

**No. at risk**

Pharmacogenomic arm	448	423	406	311
Standard of care arm	440	420	385	319

Supplementary Appendix

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Appendix A - List of Investigators

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Participating Centres

- Division of Cardiology, Ospedale Infermi di Rimini, Rimini, Italy
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- Division of Cardiology, Azienda Territoriale di Modena, Ospedale Ramazzi di Carpi, Carpi, Italy
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Principal Investigator: Dr. Massimo Pantaleoni
Sub-Investigator: Dr. Giovanni Tortorella

Participating Centre Nos. 8-12 did not receive formal ethical approval because enrolment was prematurely interrupted by the Ethics Committee of Modena.

Appendix B - Supplementary Methods

The ST Q3 instrument

The ABCB1 3435 (7q21.1; rs1045642), CYP2C19*2 (10q24.1-q24.3; rs4244285) and CYP2C19*17 (10q24.1-q24.3; rs12248560) genetic variants were genotyped using the ST Q3 system, a compact platform enabling the laboratory analysis of DNA by means of real-time PCR that has been developed by ST Microelectronics and the Division of Cardiology of Azienda Ospedaliera Universitaria di Parma. As genotyping to select P2Y₁₂ receptor antagonists in the setting of acute coronary syndromes cannot be delegated to centralised genetic laboratories for reasons of time, we designed the ST Q3 instrument for bedside genotyping as a low entry-cost portable system for foolproof use by unskilled personnel. It is used to drive a disposable lab-on-chip device consisting of a silicon die manufactured using semiconductor technology, and sealed with a transparent frame to form six chambers in which the RT-PCRs take place. (1)

DNA isolation

Peripheral blood (up to 5 mL) was drawn into Na⁺-EDTA vacuettes and transferred into a laminar flow hood in order to avoid any genomic DNA contamination. The Maxwell16™ platform (Promega) was used for DNA isolation. An aliquot (0.4 mL) of blood was transferred into a loading cartridge and the instrument isolated the DNA automatically in an average time of 28 minutes.

Genotyping

The genotyping procedure was performed using the ST Q3 instrument. The user only has to add 5 µL of DNA through a simple loading system, after which the disposable device self-seals in order to prevent cross-contamination between the different reaction chambers. After switching on the instrument and launching the dedicated Q3 controlling software, the user is guided

through easy operational steps by the software interface and dedicated videos. The reaction lasts approximately 40 minutes, after which the software displays the SNP score and the patient's genetic profile.

All of the results were automatically saved in a .pdf report file in order to ensure accurate data collection.

Workflow

The total workflow of the genotyping process consists of: 1) peripheral blood withdrawal (up to 5 mL), 2) DNA isolation using the Maxwell16™ platform, 3) loading the purified DNA into the ST lab-on-chip, 4) insertion of the chip in the ST Q3 instrument, 5) analysis of the sample and collection of the displayed results.

Workflow for the selection of P2Y12 antagonists in the pharmacogenomic arm

The patients randomised to the pharmacogenomic arm received one of the P2Y12 receptor antagonists (clopidogrel, prasugrel or ticagrelor) on the basis of their genotype (Supplementary Appendix Figure 1) in addition to clinical variables. It is important to underline that the genetic algorithm was designed to consider the three genes simultaneously, but the ultimate decision concerning antiplatelet therapy also took account the patient's clinical characteristics and was left to the discretion of the prescribers.

Unless medically contraindicated, it was suggested that clopidogrel should be administered to patients whose genetic test results indicated :

- ABCB1 3435 wild-type or heterozygous, CYP2C19*2 and CYP2C19*17 both wild-type;
- ABCB1 3435 wild-type or heterozygous, CYP2C19*2 homozygous or heterozygous, and CYP2C19*17 homozygous.

Because of the high risk of bleeding, it was suggested that prasugrel or ticagrelor should be administered to patients whose genetic test results indicated:

- ABCB1 3435 wild-type or heterozygous, CYP2C19*2 wild-type, and CYP2C19*17 homozygous.

Because of the high ischemic risk, it was suggested that prasugrel or ticagrelor should be administered to patients whose genetic test results indicated :

- ABCB1 3435 homozygous, and any CYP2C19*2 and CYP2C19*17 genotype;
- ABCB1 3435 wild-type or heterozygous, CYP2C19*2 wild-type, and CYP2C19*17 homozygous or heterozygous;
- ABCB1 3435 wild-type or heterozygous, CYP2C19*2 heterozygous, and any CYP2C19*17 genotype;
- ABCB1 3435 wild-type or heterozygous, CYP2C19*2 homozygous, and CYP2C19*17 wild-type or heterozygous.

The clinical variables used to individualise the choice of P2Y₁₂ receptor antagonist were age, weight, ischemic risk, bleeding risk, diabetes, prior history of stroke/TIA or intracranial bleeding, history of bleeding, active bleeding, anemia, chronic kidney disease.

Given the TRITON-TIMI-38 trial findings and the exclusion criteria, prasugrel was not prescribed for: 1) patients whose coronary anatomy was unknown; 2) those at high bleeding risk (particularly those with a history of stroke or TIA, those aged >75 years, and those with a body weight of <60 kg); 3) those receiving fibrinolytic therapy within the previous 24 hours; 4) those with active internal bleeding; 5) those with severe liver disease; 6) those taking oral anticoagulant therapy that could not be stopped; or 7) those with known clinically important thrombocytopenia (2).

Given the PLATO trial exclusion criteria, ticagrelor was contraindicated in: 1) patients with active pathological bleeding; 2) those with a history of intracranial bleeding; 3) those requiring dialysis, 4) those taking oral anticoagulant therapy that could not be stopped; 5) those with known clinically important thrombocytopenia; 6) those receiving fibrinolytic therapy within the previous 24 hours; and 7) those taking concomitant therapy with strong CYP3A inhibitors or inducers (3).

Education and on-site training

This was a multicentre study that was funded by the Emilia Romagna Region (Project Identification Number: E35E09000880002) and performed at the Cardiological Divisions of the participating centres. Project implementation was preceded by a preliminary 2-year phase during which the engineers, clinicians, biotechnologists and geneticists working at the Cardiology Division of Parma University Hospital and at ST Microelectronics developed, produced and validated the Q3 instrument, which provides genetic information directly and immediately at a patient's bedside.

All of the investigators were trained in the study procedures (DNA extraction, genotyping, randomisation, e-CRF data entry) during the Investigators' Meeting that took place at Parma Hospital in June 2013. Before the beginning of the study, an Initiation Visit was performed at each participating centre during which a multidisciplinary team of cardiologists and geneticists delivered the study materials to each local investigator (DNA extraction system, pipettes (10-200 μ L, 100-1000 μ L), aerosol-resistant pipette tips, ST lab-on-chips, an ST Q3 instrument and a PC with Q3 Software installed) and flow-charts showing DNA isolation, genotyping and randomisation. The members of the expert team also installed and funzionalised each

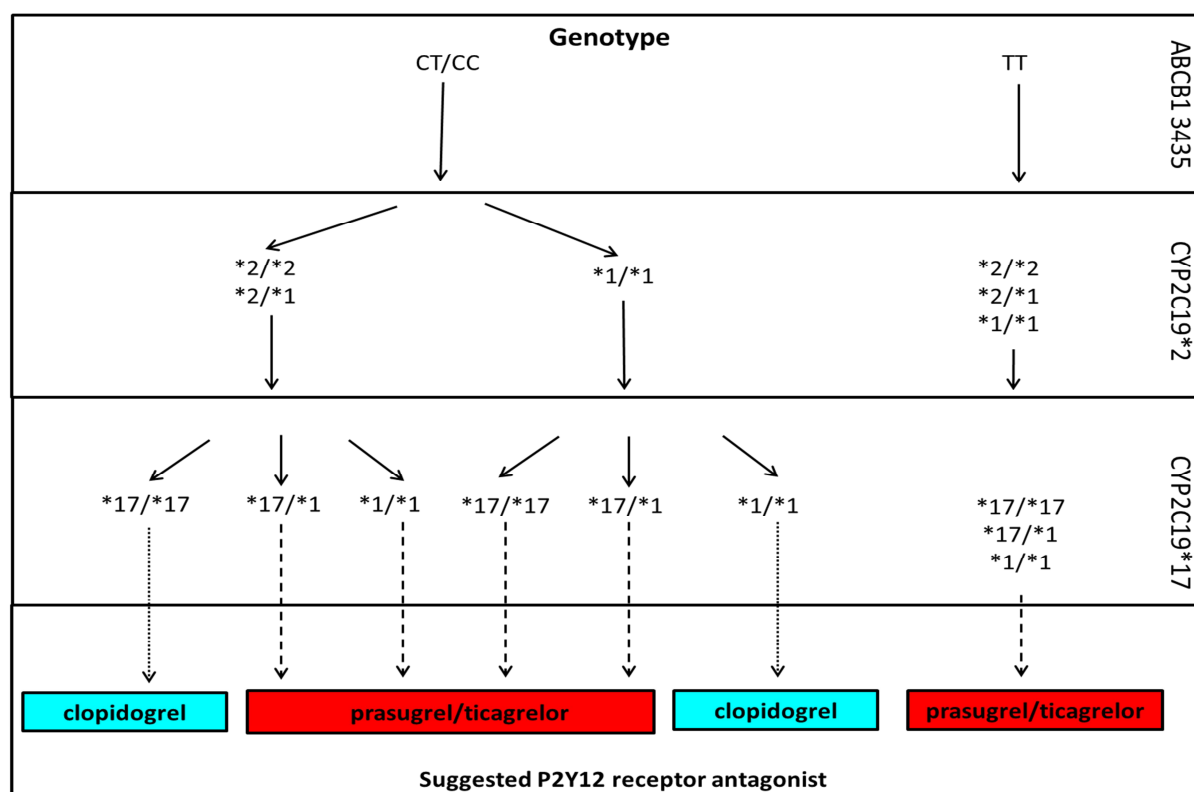
instrument, and supervised the investigators during simulations of DNA isolation and genotyping, in order to consolidate their newly-acquired skills.

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Online Figure 1. Genetic algorithm for the suggested P2Y₁₂ receptor antagonists

In ABCB1 homozygous patients, it was suggested to administer prasugrel or ticagrelor regardless of the CYP2C19*2 and CYP2C19*17 genotype. In ABCB1 heterozygous or wild-type patients, it was suggested to administer clopidogrel if they are wild type for both CYP2C19*2 and CYP2C19*17, or if they were CYP2C19*2 homozygous or heterozygous and CYP2C19*17 wild-type. For patients with all the remaining CYP2C19*2 and CYP2C19*17 genotype combinations, it was suggested to administer prasugrel or ticagrelor.



Appendix C – Follow-up and end-point definitions

Follow-up

The trigger events during follow-up were identified at the 12-month visit or by means of telephone contacts using a common data collection form. When a trigger event was recorded, the investigators provided source documents to the Clinical Event Committee. The cause of death was attributed by checking the death certificate.

All of the events were adjudicated by the independent Clinical Event Committee, which consisted of three cardiologists who were unaware of the patients' study group assignment. Two of the cardiologists reviewed the medical records and, in the case of disagreement, the opinion of a the third was required.

End-point definitions

The primary end-point was the composite of cardiovascular death, and the first occurrence of non-fatal myocardial infarction, non-fatal stroke, and BARC 3 to 5-defined major bleeding within 12 months of randomisation. The secondary end-point was the composite of the first occurrence of the primary end-point plus the occurrence of definite or probable stent thrombosis.

Cardiovascular death

All reported deaths were recorded and adjudicated. Cardiovascular death was defined on the basis of the patient's death certificate, and included sudden cardiac death, death due to acute myocardial infarction, death due to heart failure, death due to a cerebrovascular event, death due to other cardiovascular causes (i.e. pulmonary embolism, aortic disease, cardiovascular intervention)

Myocardial infarction

Myocardial infarction was defined in accordance with the universal definition (1). Any one of the following meets the diagnostic criteria for myocardial infarction.

1. The detection of a rise and/or fall in cardiac biomarkers (preferably troponin) with at least one value above the 99th percentile of the upper reference limit (URL), together with evidence of myocardial ischemia with at least one of the following:

- Symptoms of ischemia;
- ECG changes indicative of new ischemia (new ST-T changes or new left bundle branch block [LBBB]);
- Development of pathological ECG Q waves;
- Imaging evidence of a new loss of viable myocardium or a new regional wall motion abnormality.

2. The occurrence of sudden, unexpected cardiac death involving cardiac arrest, often with symptoms suggesting myocardial ischemia, and accompanied by presumably new ST elevation, or a new LBBB, and/or coronary angiographic or autopsy evidence of a fresh thrombus with death occurring before blood samples could be obtained or before the appearance of cardiac biomarkers in the blood.

3. In the case of patients undergoing a percutaneous coronary intervention (PCI) with normal baseline troponin values, elevations of cardiac biomarkers above the 99th percentile URL were considered as being indicative of peri-procedural myocardial necrosis. By convention, increases in biomarkers of $>3 \times 99^{\text{th}}$ percentile URL were designated as defining PCI-related myocardial infarction.

4. In the case of patients undergoing coronary artery bypass grafting (CABG) with normal baseline troponin values, elevations of cardiac biomarkers above the 99th percentile URL were

considered as being indicative of peri-procedural myocardial necrosis. By convention, increases in biomarkers of $>5 \times 99^{\text{th}}$ percentile URL plus new pathological Q waves or a new LBBB, or angiographically documented new graft or native coronary artery occlusion, or imaging evidence of a new loss of viable myocardium were designated as defining CABG-related myocardial infarction.

5. Pathological findings of an acute myocardial infarction.

Stroke

Stroke (2) was defined as an acute episode of neurological dysfunction attributable to a central nervous system vascular cause. Stroke had to be documented by a CT scan or magnetic resonance imaging [MRI], or autopsic evidence. When possible, stroke was classified as:

- Primary ischemic stroke, defined as an acute episode of focal brain, spinal, or retinal dysfunction caused by an infarction of central nervous system tissue and documented by imaging.
- Primary hemorrhagic stroke, defined as an acute episode of focal or global brain, spinal or retinal dysfunction caused by non-traumatic intraparenchymal, intraventricular, or subarachnoid hemorrhage, as documented by neuroimaging or autopsy. Microhemorrhages (<10 mm) evident only on MRI were not considered to be a hemorrhagic stroke.

BARC major bleeding

We defined major bleeding as BARC (Bleeding Academic Research Consortium) categories 3-5 (3):

BARC 3:

- overt bleeding plus a decrease in hemoglobin levels of 3 to <5 g/dL and/or any transfusion with overt bleeding (BARC 3a);
- or overt bleeding plus a decrease in hemoglobin levels of ≥ 5 g/dL and/or cardiac tamponade and/or bleeding requiring surgical intervention for control (excluding dental/nasal/skin/hemorrhoid bleeding) and/or bleeding requiring intravenous inotropes (BARC 3b);
- or intracranial hemorrhage (does not include microbleeds or hemorrhagic transformation; does include intraspinal bleeding), confirmed by autopsy or imaging or lumbar puncture and/or intra-ocular bleeding compromising vision (BARC 3c).

BARC 4:

- CABG-related bleeding within 48 hours.

BARC 5:

- Fatal bleeding that directly causes death with no other explainable cause. BARC fatal bleeding is categorised as either *definite* or *probable* as follows:

- Type 5a

Definite fatal bleeding is bleeding that is directly observed or confirmed on autopsy or imaging.

- Type 5b

Probable fatal bleeding is bleeding that is clinically suspicious as the cause of death, but the bleeding is not directly observed and there is no autopsy or confirmatory imaging. The site of fatal bleeding is specified as intracranial, gastrointestinal, retroperitoneal, pulmonary, pericardial, genito-urinary, or other.

Stent thrombosis

Stent thrombosis was evaluated using the Academic Research Consortium criteria (4). Definite stent thrombosis was defined as the presence of angiographic or pathological confirmation of partial or total thrombotic occlusion within the peri-stent region and at least one of the following additional criteria: acute ischemic symptoms; ischemic ECG changes; elevated cardiac biomarkers. The definition of probable stent thrombosis required the presence of an unexplained death within 30 days of the procedure or an acute myocardial infarction involving the target vessel territory without angiographic confirmation.

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