ESC European Society of Cardiology

European Heart Journal (2020) **0**, 1–10
doi:10.1093/eurheartj/ehz917

Effects of clopidogrel vs. prasugrel vs. ticagrelor on endothelial function, inflammatory parameters, and platelet function in patients with acute coronary syndrome undergoing coronary artery stenting: a randomized, blinded, parallel study

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Received 9 March 2019; revised 13 August 2019; editorial decision 12 November 2019; accepted 5 December 2019

Aims In a randomized, parallel, blinded study, we investigate the impact of clopidogrel, prasugrel, or ticagrelor on peripheral endothelial function in patients undergoing stenting for an acute coronary syndrome.

Methods and results The primary endpoint of the study was the change in endothelium-dependent flow-mediated dilation (FMD) following stenting. A total of 90 patients (age 62 ± 9 years, 81 males, 22 diabetics, 49 non-ST elevation myocardial infarctions) were enrolled. There were no significant differences among groups in any clinical parameter. Acutely before stenting, all three drugs improved FMD without differences between groups ($P = 0.73$). Stenting blunted FMD in the clopidogrel and ticagrelor group (both $P < 0.01$), but not in the prasugrel group. During follow-up, prasugrel was superior to clopidogrel [mean difference 2.13, 95% confidence interval (CI) 0.68–3.58; $P = 0.0047$] and ticagrelor (mean difference 1.57, 95% CI 0.31–2.83; $P = 0.0155$), but this difference was limited to patients who received the study therapy 2 h before stenting. Ticagrelor was not significantly superior to clopidogrel (mean difference 0.55, 95% CI -0.73 to 1.82; $P = 0.39$). No significant differences were seen among groups for low-flow-mediated dilation. Plasma interleukin (IL)-6 ($P = 0.02$ and $P = 0.01$, respectively) and platelet aggregation reactivity in response to adenosine diphosphate ($P = 0.002$ and $P = 0.035$) were lower in the prasugrel compared to clopidogrel and ticagrelor group.

Conclusion As compared to ticagrelor and clopidogrel, therapy with prasugrel in patients undergoing stenting for an acute coronary syndrome is associated with improved endothelial function, stronger platelet inhibition, and reduced IL-6 levels, all of which may have prognostic implications. This effect was lost in patients who received the study medication immediately after stenting.

EUDRACT-N° 2011-005305-73

Keywords Endothelium • Thrombosis • Acute coronary syndromes • Stent

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Introduction

Previous studies have shown that coronary artery stenting is associated with an immune/inflammatory response[1–7] and with impaired coronary and peripheral endothelial responsiveness. Although the mechanisms of this phenomenon remain unclear, there is evidence that these inflammatory reactions caused by the mechanical trauma may lead to endothelial dysfunction[8,9] and may trigger adverse vascular reactions, including in-stent restenosis and in-stent thrombosis.[10] Importantly, the degree of endothelial dysfunction early after stenting can be used as a predictor for long-term patient outcome.[11,12]

A cross-talk between platelet function and endothelial function also exists. Particularly in the setting of acute coronary syndromes (ACS), platelet activation and the mechanical injury induced by stenting cause the release of mediators of inflammation and oxidative stress, which stimulate leucocyte chemotaxis, platelet aggregation, and endothelial dysfunction (reviewed in ref.[13]). These phenomena appear to be attenuated, or interrupted, by platelet inhibition with GpIIbIIIA inhibitors.[14] Among $P2Y_{12}$ receptor inhibitors, both clopidogrel and ticagrelor have been shown to improve endothelial function in patients with stable coronary artery disease, an effect that was however lost during prolonged administration in the case of clopidogrel.[15–17] It remains unclear whether differences among $P2Y_{12}$ inhibitors exist. In a recent randomized cross-over study, ticagrelor did not improve arteriolar vessels endothelial function of when compared with prasugrel and clopidogrel in stable patients with a history of ACS.[18] In contrast, in another randomized trial in diabetic patients with non-ST elevation myocardial infarction (NSTEMI), ticagrelor significantly decreased inflammatory cytokines [interleukin (IL)-6 and tumour necrosis factor-alpha (TNF-α)], it increased circulating endothelial progenitor cells, and it improved arterial endothelial function of conduit vessels.[19]

The present study was designed to investigate the impact of antiaggregants on endothelial function as assessed by conduit artery flow-mediated dilation (FMD), platelet inhibition, and inflammatory biomarkers in a randomized, blinded fashion in a group of patients undergoing stenting (percutaneous coronary intervention, PCI) in the setting of an ACS.

Methods

Objectives of the study

The primary endpoint of the trial was the change in FMD at 1 day, 1 week, and 1 month after PCI across patients randomized to be treated with clopidogrel, prasugrel, or ticagrelor. Secondary objectives were to test the impact of the three study medications on parameters of endothelial function after an acute loading dose of the three study drugs and to investigate the changes in low-flow-induced vasoconstriction (L-FMC), as well as markers of platelet activation and inflammation/oxidative stress in the three groups. Furthermore, the safety and tolerability of clopidogrel, prasugrel and ticagrelor were investigated.

Study design

The study protocol is published in Schnorbus *et al.*[20] In brief, the study was designed as a three-arm, parallel design, randomized, investigator-blinded study. Patients with unstable angina or NSTEMI undergoing coronary intervention with a drug-eluting stent (Xience Prime, Abbott Vascular) were randomized to receive clopidogrel, prasugrel, or ticagrelor followed by oral therapy with the same drug. A list of the inclusion and exclusion criteria is presented in Supplementary material online, *Table 1S*. The randomization was carried out in a 1:1:1 ratio using block randomization without stratification by means of an SAS programme. Study drugs were prepared and packaged in identical anonymous boxes by the hospital pharmacy. All study personnel was blinded to the allocation group. The patients and treating physicians were informed on the allocation group only after the final (1 month) visit.

Trial schedule

The trial schedule is presented in *Figure 1*. In the original protocol, participants underwent six visits. Vascular function, platelet function, and markers of inflammatory stress were measured at screening, 2 h after the loading dose (only for patients recruited before amendment 1), and 1 day, 1 week, 1 month after stenting. Randomization was performed, and the study medication was administered, at the end of the screening visits. The trial schedule was amended on 24 February 2014 following the November 2013 warning by the European Medicines Agency (EMA/90532/2014) recommending the administration of prasugrel only at the time of coronary intervention. Following this recommendation, the protocol was amended so that randomization was performed (and the study medication was administered) immediately following PCI. As a consequence, the 2-h visit was eliminated. To test whether this had an impact on the study results, the timing of administration of the study medications (before vs. after angiography and percutaneous intervention) was introduced in the analysis.

Trial therapy

Study drugs were administered orally in the clinically indicated dosage including a single loading of 600 mg (clopidogrel), 60 mg (prasugrel), or 180 mg (ticagrelor) followed by chronic treatment: clopidogrel 75 mg o.d., 10 mg o.d. prasugrel, or 90 mg b.i.d. ticagrelor.

Assessment of endothelial function

Endothelial function was assessed using FMD and low-flow-mediated constriction, L-FMC), respectively reflecting endothelial responsiveness and endothelial activity at rest.[21–24] Flow-mediated dilation and L-FMC were measured using a GE vivid 7 ultrasound machine with a 14 MHz matrix linear-array transducer and automatic analysis software as previously described.[24,25] In brief, the radial artery was imaged at rest for 1 min using high-resolution ultrasound imaging. Subsequently, a pneumatic cuff was inflated distal to the imaged artery to cause a temporary (4.5′) interruption of blood flow. During distal ischaemia, the decrease in blood flow at the level of the radial artery is associated with a decrease in radial artery diameter (L-FMC). After release of the ischaemia, the sudden increase in blood flow and shear stress results in an increase in endothelium-dependent radial artery FMD. The analysis was performed off-line in a random order by staff blinded to the allocation group. The analysis of endothelial function data was performed customized software (SpLiNeS, Siena, Italy[26]) by personnel blinded to the allocation group.

Platelet function and biomarkers

Platelet reactivity testing *in vitro* was assessed by adenosine diphosphate (ADP)-induced aggregation in hirudin-anticoagulated whole blood by using whole blood impedance/multiple electrode aggregometry (Multiplate® analyser and ADPtest, Roche Diagnostics, Rotkreuz, Switzerland).[27] Concentrations of plasma sE-Selectin, sVCAM-1, sCD40 Ligand, IL-6, MCP-1, and RANTES were determined by ELISA (R&D Diagnostics, Wiesbaden, Germany) according to the manufacturer's instructions.

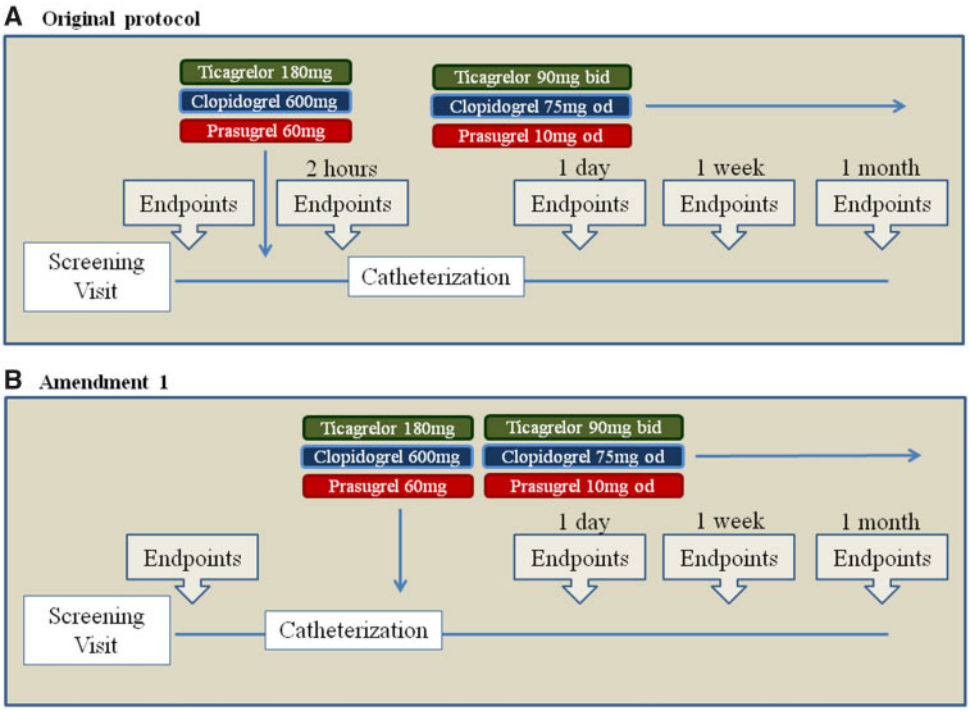


Figure 1 Study protocol before and after amendment.

Statistics

Per protocol, the efficacy analysis was based on a modified intention to treat analysis set, containing all patients who received at least one stent and had at least one FMD measurement during follow-up.

Patient characteristics are presented as mean ± standard error or number (%) as appropriate. For the primary analysis, FMDs in the prasugrel and ticagrelor groups were tested separately in two independent mixed models in which an unstructured covariance matrix incorporated the dependency between the three repeated measurements after stenting. The denominator degrees of freedom for the tests of fixed effects were computed by the Satterthwaite method. Prasugrel and ticagrelor were compared to the reference clopidogrel at a local one-sided level of alpha = 0.0125, which corresponds to a global two-sided level of alpha = 0.05, by virtue of Bonferroni adjustment. For both comparisons vs. the reference clopidogrel, a two-armed adaptive design was set to control one-sided level of alpha = 0.0125. At the request of the sponsor, an interim analysis was conducted after the first 56 patients were enrolled. The investigators remained blinded to the results of this analysis.

Details of the statistical analysis of the data collected in this trial were documented in a Statistical Analysis Plan (SAP) that was generated by the trial statistician and finalized before closing the database and breaking the randomization code. The SAP was based on the protocol including all amendments. The statistical analysis was conducted by means of SAS® or SPSS®.

Ethic committee and regulatory approval

The trial was carried out in keeping with legal and regulatory requirements and the protocol was approved by the local ethics committee and by the national authorities. National regulations (Arzneimittelgesetz and the Federal Data Protection Law) were kept. The trial was registered in ClinicalTrials.gov (Identifier: NCT01700322) and EUDRACT-N°.: 2011-005305-73. One hundred percent of the data was externally monitored by the IZKS, which was also responsible for aspects of patient safety and compliance monitoring, auditing, regulatory.

Results

Patient characteristics

A total of 90 patients (age 62 ± 9 years, 81 males, 22 diabetics) were enrolled in the study. The clinical presentation was NSTEMI in 49 patients, unstable angina in the remaining 41 patients. Patient characteristics and other medications are described in *Table 1* and Supplementary material online, *Tables 2S–4S*. There were no significant differences among groups. As well, no differences existed between patients included with the original protocol and those included thereafter (amendment 1) as shown in Supplementary material online, *Table 2S*. Procedural success (residual stenosis <10% without ischaemia) was achieved in all cases.

Efficacy analysis—endothelial function

Fifty-six patients were enrolled with the original protocol and satisfied the above criteria for the efficacy analysis. After amendment 1, 34 additional patients were included (the study was interrupted at 98% of the initially planned sample size for slow recruitment).

Treatment effects on FMD and L-FMC are displayed in *Tables 2 and 3* and Supplementary material online, *Table 5S* as well as *Figure 2 and* Supplementary material online, *Figures 1S and 2S*. Flow-mediated dilation was not different across groups at screening. After the first

Table 1 Patient characteristics

	Treatment group						P-value
	Clopidogrel		Prasugrel		Ticagrelor		
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	
Age	31	62.2 ± 10.3	27	60.6 ± 7.8	33	60.5 ± 9.0	0.7140
Clinical presentation, NSTEMI, *n* (%)		18 (58%)		14 (52%)		17 (52%)	0.8893
BMI	31	27.7 ± 4.0	27	28.1 ± 3.1	33	29.2 ± 4.3	0.2956
Cholesterol (mg/dL)	31	198 ± 45	23	208 ± 62	28	223 ± 53	0.6225
HDL-cholesterol (mg/dL)	30	45 ± 11	23	45 ± 10	28	45 ± 12	0.4427
LDL-cholesterol (mg/dL)	30	119 ± 34	23	125 ± 43.8	28	143 ± 42	0.5658
Triglycerides (mg/dL)	30	161 ± 71	23	235 ± 179	28	193 ± 140	0.6688
Creatinine (mg/dL)	31	1.0 ± 0.2	27	0.9 ± 0.2	33	0.9 ± 0.1	0.1801
Haemoglobin (g/dL)	31	15.2 ± 1.4	27	15.1 ± 1.3	33	14.8 ± 1.1	0.9912
Creatinine after stent (mg/dL)	29	0.9 ± 0.2	27	0.9 ± 0.2	33	0.9 ± 0.2	0.4126
Systolic blood pressure (mmHg)	31	137 ± 16	27	136 ± 19	33	141 ± 22	0.8744
Diastolic blood pressure (mmHg)	31	81 ± 9	27	82 ± 13	33	83 ± 13	0.4801
Comorbidities							
Diabetes		5 (16%)		5 (19%)		12 (36%)	0.1329
Hypercholesterolaemia		15 (48%)		18 (67%)		18 (55%)	0.4003
Hypertension		13 (42%)		14 (52%)		19 (58%)	0.4714
Procedural data							
Number of vessels diseased		2 (1)		2 (1)		2 (1)	0.330
Number of vessels treated		1.1		1.1		1.1	0.484
Number of stents implanted		1.2 (0.1)		1.4 ± 0.1		1.5 ± 0.1	0.111
Stent diameter (mm)		3.1 ± 0.1		3.2 ± 0.1		3.1 ± 0.1	0.585
Total stent length (mm)		21 ± 2		21 ± 2		22 ± 1	0.500

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NSTEMI, non-ST elevation myocardial infarction; SD, standard deviation.

administration (analysis limited to patients included with the original protocol), FMD improved in all groups (deltas: 1.5% for clopidogrel, 1.9% for prasugrel, 0.8% for ticagrelor, $P = 0.727$). At 1 day after PCI, FMD was decreased in the clopidogrel (delta: -3.8%, $P = 0.008$ compared to screening), and ticagrelor (delta: -3.5%, $P < 0.001$), but not in the prasugrel group (delta: -0.7%, $P = 0.734$). For the primary endpoint (FMD after PCI), prasugrel administration was associated with a significant improvement as compared to both clopidogrel [mean difference 2.13, 95% confidence interval (CI) 0.68–3.58; $P = 0.0047$] and ticagrelor (mean difference 1.57, 95% CI 0.31–2.83; $P = 0.0155$). The difference between ticagrelor and clopidogrel was not significant (0.55%, 95% CI -0.73 to 1.82; $P = 0.39$). These differences were maintained in an analysis including only NSTEMI patients ($P = 0.0013$, Supplementary material online, *Figure S3A*), while they were not present in patients with unstable angina ($P = 0.56$, Supplementary material online, *Figure S3B*). Comparisons for each visit are presented in *Table 2*. Ticagrelor was not significantly superior to clopidogrel (mean difference 0.55, 95% CI -0.73 to 1.82; $P = 0.39$). Also, there was an interaction between effect and timing of admin istration, whereby both comparisons yielded significant differences before (prasugrel vs. clopidogrel and prasugrel vs. ticagrelor; respectively $P = 0.0002$ and $P = 0.0036$) but not after (respectively, $P = 0.9979$ and $P = 0.5031$) amendment 1 (Supplementary material online, *Table 5S*).

No significant differences between the groups were seen either before or after amendment in L-FMC (*Table 3*, Supplementary material online, *Figure 2S*: mean-difference between prasugrel and clopidogrel: 0.13, 95% CI -0.96 to 1.22; $P = 0.81$; mean difference between ticagrelor and clopidogrel: -0.43, 95% CI -1.58 to 0.71; $P = 0.45$; mean difference between prasugrel and ticagrelor: 0.59, 95% CI -0.57 to 1.75; $P = 0.31$).

Plasma biomarkers

Plasma biomarkers were assessed only in the first cohort (original protocol). Data are presented in Supplementary material online, *Tables 6S–11S*. In brief, baseline values were not different across groups except for sE-Selectin levels, which were lower in the prasugrel group. After PCI, IL-6 levels were lower in the prasugrel group as compared to clopidogrel and ticagrelor ($P = 0.019$ and 0.013, respectively, *Figure 3*). A similar trend was shown by sE-selectin, although these differences did not reach significance ($P = 0.053$ at visit 3). Otherwise, neither PCI nor the drugs administered induced any change in plasma biomarkers. As compared to those with unstable

Table 2 **Endothelial function data—FMD**

FMD (%)										
Measurement time	**Treatment group**			**Comparisons averaged over time**			**Within-visit comparisons**			
	Clopidogrel (*n* = 31)	**Prasugrel (*n* = 27)**	**Ticagrelor (*n* = 33)**	**P vs. C**	**P vs. T**	**T vs. C**	**Overall effect**	**P vs. C**	**P vs. T**	**T vs. C**
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Model-based contrast estimates (95% CI), *P*-value				Mean difference (95% CI), *P*-value		
Screening	4.40 (3.05–5.75)	4.29 (2.84–5.74)	3.81 (2.50–5.12)							
2 h after first dose	5.91 (4.69–7.13)	5.33 (3.96–5.70)	6.37 (5.15–7.59)							
1 day	2.34 (1.16–3.52)	4.56 (3.31–4.81)	2.89 (1.73–4.05)	2.13 (0.68–3.58) *P* = 0.0047	1.57 (0.31–2.83) *P* = 0.016	0.55 (-0.73 to 1.82) *P* = 0.39	*P* = 0.023	2.44 (0.50–4.38) *P* = 0.015	1.64 (0.04 to 3.24) *P* = 0.045	0.80 (-0.98 to 2.58) *P* = 0.37
6 days	3.19 (1.92–4.46)	4.99 (3.68–6.30)	3.07 (1.82–4.32)				*P* = 0.017	2.01 (0.23–3.79) *P* = 0.027	2.66 (0.70 to 4.63) *P* = 0.0089	0.65 (-1.00 to 2.28) *P* = 0.43
28 days after stenting	2.78 (1.72–3.84)	5.11 (3.80–6.42)	3.98 (2.72–5.25)				*P* = 0.039	2.32 (0.42–4.22) *P* = 0.018	1.47 (-0.26 to 3.20) *P* = 0.094	0.85 (-0.96 to 2.66) *P* = 0.35

Overall effects were compared with one-way analysis of variance (ANOVA).
C, Clopidogrel; P, Prasugrel; T, Ticagrelor.

Table 3 Endothelial function data—L-FMC

L-FMC (%)						
	Treatment group			Model-based contrast estimates averaged over time		
	Clopidogrel ($n = 31$)	Prasugrel ($n = 27$)	Ticagrelor ($n = 33$)			
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	P vs. C	P vs. T	T vs. C
Measurement time				Effect estimate (95% CI), *P*-value		
Screening	-2.71	-4.26	-3.26			
	(-1.42 to -4.0)	(-2.89 to -5.63)	(-2.01 to -4.51)			
2 h after first dose	-3.59	-5.22	-2.33			
	(-2.32 to -4.86)	(-2.77 to -6.67)	(-1.06 to -3.60)			
1 day	-4.08	-4.67	-4.87	0.13	0.59	-0.43
	(-2.96 to -5.20)	(-3.47 to -5.87)	(-3.77 to -5.97)	(-0.96 to 1.22)	(-0.57 to 1.75)	(-1.58 to 0.71)
6 days	-3.93	-3.50	-3.42	$P = 0.813$	$P = 0.312$	$P = 0.451$
	(-2.71 to -5.15)	(-2.23 to -4.77)	(-2.22 to -4.62)			
28 days after stenting	-3.89	-3.29	-4.91			
	(-2.83 to -4.95)	(-2.17 to -4.41)	(-3.83 to -5.99)			

C, Clopidogrel; P, Prasugrel; T, Ticagrelor.

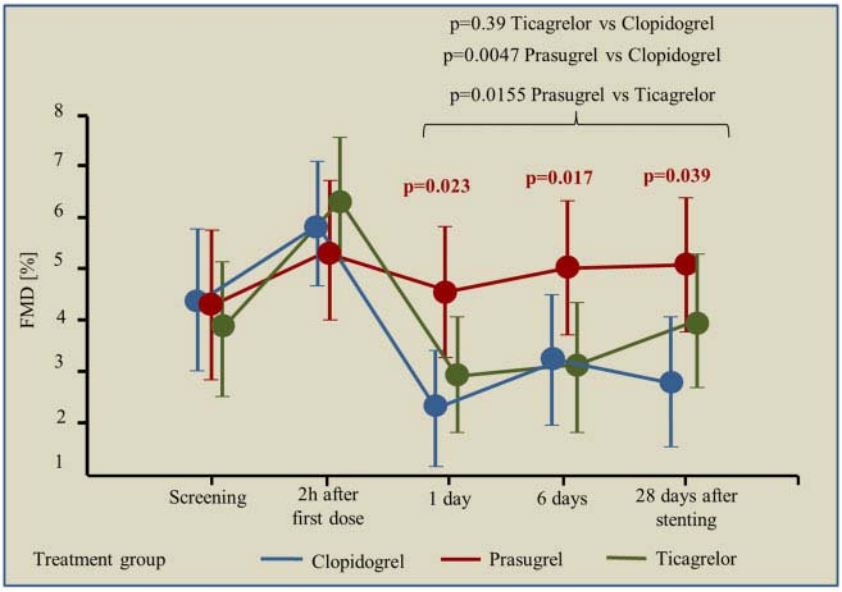


Figure 2 The impact of the three study medications on flow-mediated dilation. Percutaneous coronary intervention impaired flow-mediated dilation in the clopidogrel and ticagrelor group, and flow-mediated dilation remained higher during follow-up in the prasugrel group.

angina, patients with NSTEMI had higher IL-6 levels at admission ($P = 0.026$) and 1 day after stenting ($P = 0.015$).

Platelet aggregatory reactivity in response to ADP

Platelet aggregation data are presented in *Table 4*. There was no difference among groups at screening. ADP-induced platelet aggregation capacity was lower in the prasugrel and ticagrelor groups at 2 h after the first administration ($P = 0.018$). At 1 day and 1 month, platelet aggregation was most inhibited in the prasugrel group ($P = 0.002$ and $P = 0.035$ among groups). Interestingly, this difference was evident in the NSTEMI patients ($P = 0.0001$), but not in those with unstable angina ($P = 0.49$), Supplementary material online, *Figure S4*.

Safety

All 125 patients who had received at least one administration of the study drug (including the 90 enrolled patients and 35 additional ones

who, before amendment 1, received medication before a negative angiography and were not randomized) were included in the safety analysis. The list of serious adverse events is presented in Supplementary material online, *Table 12S*. Sixteen (43.2%) of all adverse events were judged as mild, 15 (40.5%) as moderate, and 6 (16.2%) as severe. Two (33.3%, 0.05 per patient) of the six severe events occurred in the clopidogrel group and 4 (66.7%, 0.1 per patient) in the ticagrelor group. Twelve (32.4%, 0.1 per patient) adverse events were judged as serious according to the definition in the study protocol, none of these was assessed as related to the investigational medicinal product.

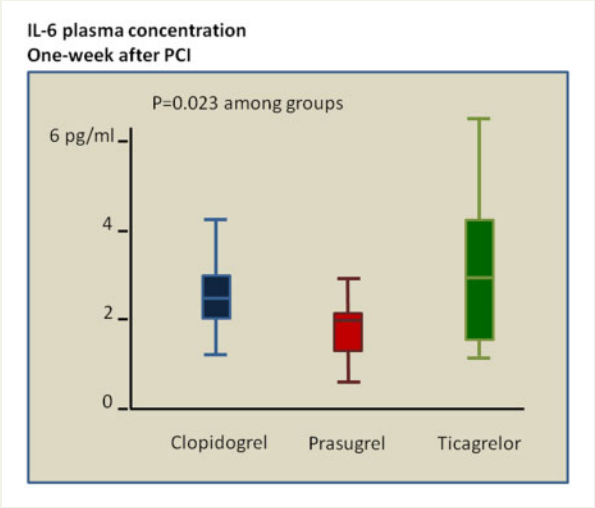


Figure 3 The impact of the three study medications on interleukin-6 plasma levels post-percutaneous coronary intervention. Levels where lowest in the prasugrel group.

Discussion

While effective in restoring perfusion to ischaemic areas, PCI provokes mechanical injury to the vascular wall and endothelium.[13] The resulting local inflammatory responses, characterized by platelet aggregation and leucocyte adhesion and infiltration, are regulated by a number of mediators, including IL-6, C-reactive protein, and other acute-phase reactants.[28] Importantly, this inflammatory response induced by stenting has been associated with in-stent restenosis and adverse long-term prognosis after PCI.[29,30] Although the mechanisms underlying this association remain unclear, it is well accepted that local/systemic inflammation may have a negative impact on endothelial function. In turn, endothelial dysfunction may compromise stent strut healing, promote thrombosis/restenosis or the progression of atherosclerosis and also has been associated with long-term prognosis.[13,30–35]

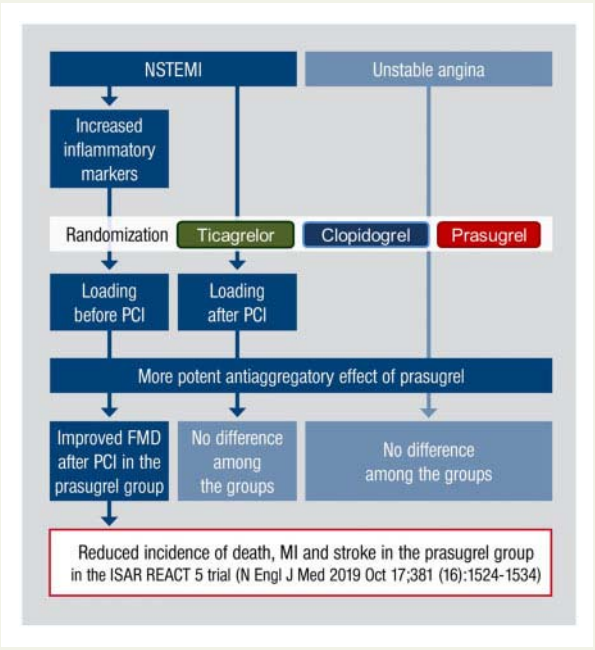
Similarly, platelets have been shown to have a role in both vascular inflammation and atherogenesis,[36] and several studies have shown that platelets are an important source of oxidative stress in ACS and in the setting of PCI.[37,38] In patients with stable angina or shortly after an ACS, antiplatelet agents improve endothelial function,[11,16,19] an effect that is however lost upon prolonged treatment with clopidogrel.[15] Less is known with regards to stent-induced vascular dysfunction.

In the present study, we compare the effect of three antiplatelet agents of different potency on markers of endothelial function, platelet function, and inflammation/oxidative stress in patients undergoing PCI for ACS. The major findings of the study (*Take home figure*) include the following: (i) acutely (before PCI), antiplatelet therapy improved FMD without a difference among drugs. (ii) On Day 1 after

Table 4 Platelet aggregation reactivity in hirudin-anticoagulated whole blood assessed by Multiplate-ADPtest

	Multiplate-ADPtest area under the curve (AU*min)						
	Clopidogrel ($n = 31$) Mean (95% CI)	Prasugrel ($n = 27$) Mean (95% CI)	Ticagrelor ($n = 33$) Mean (95% CI)	Within-visit comparisons Overall effect	Pairwise comparisons P vs. C	P vs. T	T vs. C
Screening	54 (44–64)	52 (44–60)	56 (48–64)				
2 h after first dose	36 (26–46)	21 (11–31)	23 (19–27)	$P = 0.014$	15.61 (2.15–29.07) $P = 0.024$	2.09 (-7.77 to 11.94) $P = 0.67$	13.52 (2.74 to 24.30) $P = 0.015$
1 day	15 (13–17)	8 (6–10)	12 (10–14)	$P = 0.002$	6.42 (2.72–10.11) $P = 0.001$	3.54 (0.55 to 6.54) $P = 0.021$	2.87 (-068 to 6.42) $P = 0.11$
28 days after stenting	24 (18–30)	17 (15–19)	19 (17–21)	$P = 0.035$	6.74 (0.46–13.01) $P = 0.036$	1.81 (-1.56 to 5.19) $P = 0.28$	4.92 (-0.67 to 10.53) $P = 0.084$

C, Clopidogrel; P, Prasugrel; T, Ticagrelor.



Take home figure Patients with NSTEMI or unstable angina were randomized to one of the three antiplatelet agents. Prasugrel had a more potent antiplatelet effect and was associated with an improved endothelial function before and after stenting. This effect was only present in patients treated with prasugrel before stenting. These observations might be compatible with the results of the ISAR REACT-5 trial.

PCI and during subsequent follow-up, prasugrel prevented the endothelial dysfunction associated with stenting and was a more potent inhibitor of platelet aggregation. However, this protective effect of prasugrel disappeared when the drugs were administered immediately after PCI. (iii) The more potent antiplatelet effect of prasugrel was more evident in NSTEMI patients as compared to patients with unstable angina, and NSTEMI patients showed higher levels of IL-6. Finally, (iv) PCI increased IL-6 plasma levels, an effect which was also prevented by prasugrel, while no difference in the other biomarkers was observed. Collectively, these data appear to support the link between inflammation, platelet activation, and endothelial (dys)function in ACS, and that prasugrel might be a more potent inhibitor of this cascade in the acute ACS/PCI phase. These results might concur to explain the benefit of prasugrel over ticagrelor shown in the recent ISAR-REACT 5 trial.[39]

The pleiotropic effects of $P2Y_{12}$-receptor antagonists

$P2Y_{12}$ receptor antagonists directly inhibit platelet activation and aggregation, which are involved in inflammatory processes in the development of atherosclerosis and following PCI.[40] A number of studies show that, as compared to aspirin alone, prolonged therapy with clopidogrel is associated with reductions in biomarkers like high-sensitivity C-reactive protein (hs-CRP), sCD40L, P-selectin, and IL-6.[41–46] As well, stronger platelet inhibition with prasugrel appears to lead to further reductions in inflammatory markers.[47–49] Among these markers, the proinflammatory cytokine IL-6 is involved in the genesis and progression of atherosclerosis, and elevated IL-6 plasma levels correlate with increased cardiovascular 5-year mortality independently of standard cardiovascular risk factors.[50]

Antiplatelet therapy has also been associated with improvements in endothelial function. In the Armyda trial, 150 mg clopidogrel improved FMD and reduced C-reactive protein as compared to standard 75 mg.[51] In the paper by Rudolph *et al.*,[52] prasugrel, but not clopidogrel, improved FMD and reduced sCD40 ligand and RANTES levels, while increasing nitrite levels, 3 months after PCI for unstable angina. In the paper by Jeong *et al.*,[19] prasugrel was compared to ticagrelor in a cross-over design. This study showed a larger effect of ticagrelor compared to prasugrel in terms of FMD, IL-6, TNF-α, and circulating endothelial progenitor cells, leading to the hypothesis that adenosine-mediated effects of ticagrelor might provide additional benefits. In the Hi-Tech study,[18] stable patients received the three $P2Y_{12}$ inhibitors in a cross-over design, and FMD was studied in nine of them, showing no difference among therapies. Thus, platelets are important mediators and contributors in determining inflammation, endothelial function, and ultimately patient outcome after PCI. Antiplatelet agents influence this relationship, with possible differences among drugs of different potency. In none of these studies, however, was FMD measured before PCI and before the first administration of $P2Y_{12}$ inhibitor. Of importance, the timing of administration and the clinical presentation also appear to be determinant. In our study, the positive effect of prasugrel was shown in the cohort included in the original protocol (when prasugrel was administered at least 2 h before PCI), but not in the cohort in which prasugrel was administered immediately following PCI, demonstrating that early (pre-PCI) antiplatelet inhibition may help prevent endothelial damage. Interestingly, the effect of prasugrel both in terms of platelet inhibition and endothelial function was more evident in NSTEMI patients than in unstable angina ones. Non-ST elevation myocardial infarction patients showed higher levels of IL-6 at admission, potentially supporting the concept that the effect of prasugrel on endothelial function might be mediated by (platelet-dependent) inhibition of vascular inflammation.

Limitations

This randomized, blinded study relies on a small sample size, with a large prevalence of males, and needs further replication. Given the acute nature of the clinical presentation (acute coronary syndrome and stenting), a cross-over design could not be applied to this study. Inflammatory markers were measured in the peripheral circulation, but act in a microenvironment, and some effects might have been missed. A placebo control was clearly impossible given the indication to dual antiplatelet therapy after stenting. Finally, persistent increased levels of inflammatory markers have been shown up to 12 weeks after PCI and ACS.[53,54] It is impossible to state whether the source of inflammation is the underlying disease, the PCI, or a combination of the two, and whether potent platelet inhibition might provide a benefit in either or both cases.

Conclusions

Various randomized clinical trials have shown a significant benefit of potent $P2Y_{12}$ inhibitors over clopidogrel in ACS and stable high-risk

patients. The current data show that, when administered pre-PCI, prasugrel, but not the other agents, limits stent-induced endothelial dysfunction and inflammation in ACS.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Acknowledgements

We thank Kathrin Groß for her excellent technical assistance.

Funding

This work was supported by the University Medical Centre of the Johannes Gutenberg-University Mainz. The research was funded by the German Ministry for Research through the IFB project 'Centre for Thrombosis and Hemostasis' at the University Medical Center Mainz.

Conflict of interest: T.G. reports personal fees from Lilly-Daiichi Sankyo (regional advisory board speaker's honoraria). T.G. participated (and received speaker's honoraria) in symposia and scientific sessions sponsored by Astra Zeneca and Lilly-Daiichi Sankyo. All other authors have no conflicts of interest to declare with regards to this article. T.G. and T.M. received speaker's honoraria from Abbott Vascular, which however was not involved in the study and whose products were not tested here.

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