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Biomarkers of platelet activation and cardiovascular risk in the DAPT trial

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Abstract

Prolonged use of dual antiplatelet therapy (DAPT) post-percutaneous coronary intervention (PCI) has been shown to reduce the risk of major adverse cardiovascular events (MACE), but with increased bleeding. It remains unknown whether biomarkers of platelet activation may be useful for identifying patients at increased risk of MACE. The DAPT study was a randomized trial of 12 versus 30 months of DAPT in patients who underwent PCI. Serum biomarkers [myeloid-related protein (MRP)-8/14, P-selectin, soluble CD-40 ligand (sCD40L)] were assessed in 1399 patients early post-PCI. On-treatment platelet reactivity index (PRI) using VASP phosphorylation was assessed in 443 patients randomized to continued DAPT at 1 year. MACE was defined as CV death, MI, or ischemic stroke. Multivariable models were adjusted for baseline characteristics, index event, and stent type. A stepwise increase in the risk of MACE was observed with increasing tertiles of both MRP-8/14 and P-selectin (p-trend = 0.04 for both). After multivariable adjustment,

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Ethical approval Ethics approval was obtained for all participating institutions.

Informed consent Informed consent was obtained for all participants. Patients were informed of intent to publish study results.

the adjusted HR (95% CI) for MACE in patients in the top tertile was 1.94 (1.14–3.30) for MRP-8/14 and 1.62 (0.99–2.64) for P-selectin. In contrast, baseline sCD40L was not associated with CV risk. Among patients randomized to continued DAPT, higher on-treatment platelet reactivity was not significantly associated with risk of MACE (p-trend = 0.32; adj-HR T3 vs. T1 1.54, 95% CI 0.20–12.18) or bleeding (P-trend = 0.17; adj-HR 0.25, 95% CI 0.05–1.21). MRP-8/14 and soluble P-selectin may be useful for identifying patients at increased risk of MACE after PCI. The utility of on-treatment platelet function testing requires further study.

Clinical Trial Registration—https://www.clinicaltrials.gov. Unique identifier NCT00977938.

Keywords

Platelet function testing; VASP phosphorylation; Dual antiplatelet therapy; P-selectin; MRP-8/14

Introduction

Nearly half a million inpatients undergo percutaneous coronary intervention (PCI) for obstructive coronary artery disease (CAD) in the United States each year [1]. Because of the risk of recurrent events and stent thrombosis, guidelines from cardiovascular professional societies recommend that patients receive dual antiplatelet therapy (DAPT) with aspirin and a $P2Y_{12}$ inhibitor for at least 1 year following PCI in the setting of an acute coronary syndrome (ACS) [2, 3]. In patients who complete 1 year of DAPT without suffering a cardiac ischemic or bleeding event, continued use of DAPT beyond 1 year further decreases the risk of late stent thrombosis and prevents cardiac ischemic events remote to the stented portions of the coronary artery, but increases the risk of major bleeding [4]. Given the tradeoff between attenuated cardiovascular risk and increased risk of bleeding, risk stratification is important for determining the optimal duration of DAPT following PCI [5].

Prior studies have suggested the prognostic utility of circulating serum biomarkers of platelet activation in cardiovascular risk stratification. Myeloid-related protein (MRP)-8/14, a biomarker identified through transcript profiling of activated platelets, plays a role in calcium signaling, cytoskeletal reorganization, leukocyte adhesion, and endothelial cell inflammation [6, 7]. In a cohort of apparently healthy individuals, plasma concentration of MRP-8/14 was an independent predictor of first coronary events [8]. P-selectin, an adhesion molecule stored in the alpha-granules of platelets and released into the plasma upon platelet activation [9, 10], has been associated with incident cardiovascular disease in individuals both with and without atherosclerotic risk factors [11], and with risk of recurrent arterial events in patients with prior myocardial infarction (MI) [12]. Finally, elevated soluble CD40 ligand (sCD40L), which is shed from stimulated lymphocytes and actively released from stimulated platelets, has been associated with increased risk of death and recurrent MI in patients with ACS [13].

Similarly, platelet function testing to quantify pharmaco-dynamic response to antiplatelet therapy is an established tool for cardiovascular risk assessment. One method for quantifying platelet function is platelet vasodilator-associated phosphoprotein (VASP) phosphorylation. This assay quantifies phosphorylation of an intraplatelet actin regulatory protein downstream

to activation of the $P2Y_{12}$ ADP receptor, and thus is useful for quantifying response to $P2Y_{12}$ inhibitors [14]. Patients with a reduced platelet response to clopidogrel as measured with the VASP platelet reactivity index (PRI), have been reported to have a higher incidence of cardiovascular complications, including stent thrombosis [15, 16]. Whether on-treatment platelet reactivity as measured by VASP phosphorylation can help identify patients at increased ischemic risk remains unknown.

We therefore designed a nested, prospective biomarker substudy among patients enrolled in the DAPT study to evaluate the prognostic association of circulating biomarkers of platelet activation and platelet function testing as measured by VASP phosphorylation.

Methods

Study population and design

The DAPT study was a multinational, randomized, double-blind, placebo-controlled trial designed to test the efficacy and safety of continued thienopyridine therapy (clopidogrel or prasugrel) beyond 1 year in patients taking aspirin and a thienopyridine during the first year following coronary stenting. By design, patients could enter the study de novo or through one of four post-marketing surveillance studies. Patients entering the study de novo were enrolled within 72 h of stent placement and received open-label aspirin plus thienopyridine for the first 12 months after stent implantation. At 12 months, eligible patients from either the de novo cohort or one of the post-marketing surveillance studies who were free from major bleeding and ischemic events and adherent to therapy were randomized to continued thienopyridine vs. placebo (in addition to aspirin) for an additional 18 months. After completing the randomized treatment period (i.e., 30 months post-PCI), patients were followed for an additional 3 months (i.e., 33 months post-PCI) during which they took aspirin alone.

Biomarkers and platelet function testing

Site participation in the prospective nested biomarker and platelet function substudies was voluntary and offered to all participating centers in the United States (US). Sixty sites agreed to participate and received ethics approval through an ancillary protocol. Baseline blood samples were collected for protein biomarker assessment following enrollment and shortly before or after stent placement, and serum was stored at − 20 °C or colder until shipped to the central laboratory on dry ice, where it was stored at − 70 °C or colder until thawed for analysis at the TIMI Clinical Trials Laboratory (Boston, MA). MRP-8/14 (Buhlmann Labs, Amherst, NH), P-selectin (R&D Systems, Minneapolis, MN) and sCD40L concentrations (R&D Systems, Minneapolis, MN) were measured in serum with enzyme-linked immunoassays.

On-treatment platelet reactivity was assessed using VASP phosphorylation [14]. Citrated whole blood was shipped overnight to a central laboratory (Boston Children's Hospital, Boston, MA), and VASP PRI was measured using a quantitative flow cytometry assay (BioCytex, France) [14]. Higher values indicate greater $P2Y_{12}$ receptor activation with the agonist adenosine diphosphate (ADP) and thus weaker thienopyridine-mediated $P2Y_{12}$

receptor inhibition. The VASP analyses were restricted to samples obtained at the time of the randomization visit (~ 12 months after PCI), because the timing of the baseline blood draw (trial enrollment) was not standardized relative to the timing of thienopyridine administration, and this information was not captured in the case record form. The VASP analyses were further restricted to patients allocated to continue DAPT since the on-treatment platelet reactivity may not be relevant for patients stopping thienopyridine therapy.

Clinical outcomes

The primary outcome of interest for this analysis was the cumulative incidence of major adverse cardiovascular events (MACE), defined as the composite of cardiovascular death, myocardial infarction, or ischemic stroke. Bleeding events were classified using the Bleeding Academic Research Consortium (BARC) definitions [17]. An independent clinical events committee, blinded to study assignment, adjudicated all reported outcomes for this analysis according to prespecified criteria.

Statistical analysis

Cumulative MACE event rates from trial enrollment (i.e., immediately post-PCI) through 30 months (i.e., end of randomized treatment period) were calculated for tertiles of each biomarker of platelet activation using the Kaplan–Meier method. Adjusted estimates of the association between baseline levels of each biomarker and MACE were calculated using multivariable Cox regression with the biomarker modeled as an independent variable along with age, sex, race, body mass index (BMI), diabetes mellitus, current smoking, prior stroke or transient ischemic attack, peripheral artery disease, prior MI, MI (STEMI or NSTEMI) at presentation, and stent type (paclitaxel, non-paclitaxel DES or bare metal stent). Platelet function analyses (VASP) examined cumulative MACE event rates from randomization (i.e., 12 months post-PCI) through 30 months by PRI tertile for patients randomized to continued DAPT. Adjusted estimates of the association between baseline PRI levels and MACE were calculated using multivariable Cox models with the same covariates as the serum biomarkers.

Results

Patient characteristics

Baseline characteristics of patients in the circulating serum biomarkers of platelet activation (n = 1399) and platelet function testing cohorts (n = 443) are summarized in Table 1. Baseline characteristics stratified by tertiles of MRP-8/14, P-selectin, sCD40L, and PRI are shown in Supplemental Tables 1, 2, 3 and 4 and for the full DAPT cohort in Supplemental Table 5. Increasing concentrations of MRP-8/14, P-selectin, and sCD40L were each associated with younger age and current smoking (Supplemental Tables 1, 2, 3). Higher on-treatment month 12 PRI was associated with non-white race, diabetes mellitus, and higher BMI (Supplemental Table 4).

Biomarkers of platelet activation and cardiovascular outcomes

A stepwise increase in the risk of MACE was observed by increasing tertile of MRP-8/14 (p-trend = 0.04, Fig. 1). After adjusting for baseline differences, MRP-8/14 concentration in the top tertile was associated with an increased risk of MACE through long-term follow-up [adjusted hazard ratio (adj-HR) for top (T3) vs. bottom (T1) tertile 1.94, 95% confidence interval (CI) 1.14–3.30]. Likewise, there was a significant increase in risk of MACE per SD of increasing MRP-8/14 concentration (adj-HR per SD 1.27, 95% CI 1.05–1.55).

Similarly, an increase in the risk of MACE was observed by increasing tertile of P-selectin (p-trend = 0.04, Fig. 1). After multivariable adjustment, the adj-HR for MACE was 1.62 (95% CI 0.99–2.64) for T3 vs. T1. For each SD increase in P-selectin concentration, the adj-HR for MACE was 1.19 (95% CI 0.96–1.48). Levels of sCD40L were not associated with risk of MACE (p-trend = 0.94; adj-HR for T3 vs. T1 1.09, 95% CI 0.66–1.80; adj-HR per SD 0.99, 95% CI 0.82–1.20, Fig. 1).

Among patients randomized to continued DAPT, the event rates for MACE per increasing tertile of month 12 VASP PRI were 2.10%, 3.45%, 4.27% (p-trend = 0.32) and for bleeding (BARC 2, 3 or 5) were 6.29%, 5.48%, 2.86% (p-trend = 0.17, Fig. 2). After multivariable adjustment, the adj-HR for MACE was 1.54 (95% CI 0.20–12.18) for T3 vs. T1, and the adj-HR for bleeding was 0.25 (95% CI 0.05–1.21) for T3 vs. T1 for patients with higher on-treatment platelet reactivity at randomization.

Discussion

In this nested, prospective biomarker substudy of the DAPT trial, we demonstrated that higher concentrations of MRP-8/14 and P-selectin were associated with increased risk of MACE in patients undergoing coronary stenting. Our data suggest that these biomarkers of platelet activation may be useful for identifying patients at increased risk of cardiovascular events after PCI.

Optimizing risk stratification in patients undergoing coronary stenting

Although mortality in patients with acute and chronic coronary syndromes has declined substantially over the last several decades, patients undergoing coronary stenting remain at increased risk for cardiovascular events. The improvement in outcomes has been related in large part to the expansion of medical therapies (e.g., lipid-lowering therapies, antithrombotic therapies, glucose-lowering therapies, etc.) used to treat patients with coronary heart disease. However, since these therapies may have counter-balancing risks, risk stratification is important for identifying patients at sufficiently high risk to justify their use.

The results of our analysis provide a validation of the prognostic association of MRP-8/14 and P-selectin as emerging biomarkers for identifying increased cardiovascular risk in patients undergoing coronary stenting in this well characterized clinical trial population. Although the current study did not suggest that sCD40L is a useful prognostic marker in this study population, this may in part reflect the pre-analytical issues that have been observed with the assay [18], including use of serum as opposed to plasma samples. Unfortunately,

the current study was underpowered to directly examine the question of whether these markers may be useful for identifying patients who should continue longer duration of DAPT after PCI. Nevertheless, these biomarkers did identify patients at increased risk of MACE who may therefore benefit from more aggressive medical management. Although the association between on-treatment VASP PRI and CV risk was not statistically significant, a directional trend was observed, thereby supporting prior studies that have shown an association with CV risk [15, 16]. To that end, there continues to be interest in whether platelet function testing and genotyping may be useful for helping to guide decisions about choices and duration of antiplatelet therapy.

Limitations

The platelet function and biomarker substudies were not a mandatory part of the study protocol and therefore site participation was limited. The current analysis is therefore relatively underpowered to detect smaller risk associations and could not examine treatment interactions. These questions should therefore be prospectively addressed in future studies. Furthermore, since the DAPT trial excluded patients who had an ischemic or bleeding event during the first 12 months post-PCI, patients in the VASP analysis cohort (all of whom were randomized at 12 months) may have been less likely to experience ischemic or bleeding events during follow-up as compared with a cohort of all post-PCI patients. This may have limited the power to detect associations between PRI and clinical events in this study. In addition, an analysis of platelet function and outcomes was not conducted with samples from the time of enrollment, since the timing of thienopyridine administration in relation to sample collection was not recorded. Nonetheless, these data provide insights into the prognostic utility of the selected biomarkers of platelet activation in patients undergoing coronary stenting and may be considered in future risk stratification algorithms. Additional methods for assessing platelet function testing were not compared, and sample shipping may have affected quality due to platelet activation during handling. Finally, there was no adjustment for multiple hypothesis testing in this analysis; therefore, these data should be considered exploratory and require confirmation.

Conclusions

Soluble MRP-8/14 and P-selectin, two biomarkers of platelet function, were associated with MACE in patients undergoing coronary stenting. In addition to providing pathobiological insights into residual cardiovascular risk in this patient population, these data suggest that these biomarkers may be useful for identifying patients at increased risk of cardiovascular events after PCI. Further research is needed to investigate the utility of on-treatment platelet reactivity testing for identifying patients who may benefit from more prolonged DAPT duration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflict of interest

Dr. Berg has nothing to disclose. Dr. Yeh has received Consulting Fees and Research Grants from Abbott Vascular, AstraZeneca, Boston Scientific and Medtronic. Laura Mauri is currently a Full-Time Employee of Medtronic. Dr. Morrow has received Grants and Personal Fees from Abbott Laboratories, AstraZeneca, Roche Diagnostics, and Bayer Pharma; Grants from Novartis, BRAHMS, Daiichi-Sankyo, Eisai, GlaxoSmithKline, Takeda, Pfizer, Quark, The Medicines Company, Merck, and Zora Diagnostics; Personal Fees from InCarda, Aralez, Peloton, and Verseon. He is a Member of the TIMI Study Group, for which he has received Institutional Research Grant support through Brigham and Women's Hospital from Abbott, Amgen, Aralez, AstraZeneca, Bayer HealthCare Pharmaceuticals, Inc., BRAHMS, Daiichi Sankyo, Eisai, GlaxoSmithKline, Intarcia, Janssen, MedImmune, Merck, Novartis, Pfizer, Poxel, Quark Pharmaceuticals, Roche, Takeda, The Medicines Company, and Zora Biosciences. Dr. Cutlip has received Consulting Fees from Celonova. Ms. Gao has nothing to disclose. Dr. Jarolim has received Research Grants through his institution from Abbott Laboratories, Amgen, Inc., AstraZeneca, LP, Daiichi-Sankyo, Inc., GlaxoSmithKline, Merck and Co, Inc., Roche Diagnostics Corporation, Takeda Global Research and Development Center, and Waters Technologies Corporation; and Consulting Fees from Roche Diagnostics Corporation. Dr. Michelson has received Research Grants from Ironwood Pharmaceuticals. Dr. Frelinger has received Research Grants from Ironwood Pharmaceuticals. Dr. Cange has nothing to disclose. Dr. Sabatine reports Grants from AstraZeneca during the conduct of the study; Grants and Personal Fees from Amgen, Personal Fees from Anthos Therapeutics, Grants and Personal Fees from AstraZeneca, Grants from Bayer, Personal Fees from Bristol-Myers Squibb, Personal Fees from CVS Caremark, Grants from Daiichi-Sankyo, Personal Fees from DalCor, Personal Fees from Dyrnamix, Grants from Eisai, Personal Fees from Esperion, Grants from GlaxoSmithKline, Personal Fees from IFM Therapeutics, Grants and Personal Fees from Intarcia, Personal Fees from Ionis, Grants and Personal Fees from Janssen Research and Development, Grants and Personal Fees from Medicines Company, Grants and Personal Fees from MedImmune, Grants and Personal Fees from Merck, Grants and Personal Fees from Novartis, Grants from Pfizer, Grants from Poxel, Grants from Quark Pharmaceuticals, Grants from Takeda, outside the submitted work; and is a Member of the TIMI Study Group, which has also received Institutional Research Grant support through Brigham and Women's Hospital from: Abbott, Aralez, Roche, and Zora Biosciences. Dr. O'Donoghue has received Research Grants from GlaxoSmithKline, Janssen, The Medicines Company, and Merck.

Data availability

Data requests can be made to Baim Research Institute. Code availability Data requests can be made to Baim Research Institute.

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Highlights

- The DAPT study enrolled patients at time of percutaneous coronary intervention (PCI) and randomized them at 12 months to continued dual antiplatelet therapy versus aspirin monotherapy. Markers of platelet activation were measured in a subset of participants.
- The biomarkers of platelet activation, MRP-8/14 and soluble P-selectin, were associated with a higher risk of MACE when measured after PCI.
- Higher on-treatment platelet reactivity using VASP phosphorylation suggested that higher platelet reactivity was not significantly associated with a higher risk of MACE in this patient cohort but requires further analysis in a larger patient cohort.
- These findings suggest that markers of platelet activation help to indicate patients at higher risk of MACE after PCI.
- Future directions should include whether similar markers may be useful for helping to identify patients who benefit from more prolonged dual antiplatelet therapy.

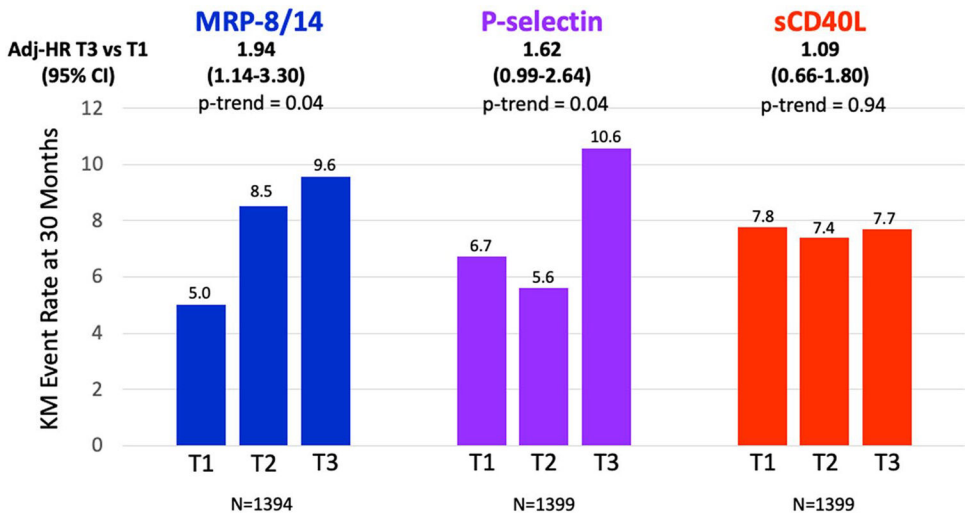

Fig. 1.

Serum biomarkers of platelet reactivity at baseline and risk of the composite endpoint of cardiovascular death, myocardial infarction, or ischemic stroke through long-term follow-up. Kaplan–Meier event rates are reported through 30 months (i.e., 18 months post-randomization). *KM* Kaplan–Meier, *MRP* myeloid-related protein, *sCD40L* soluble CD40 ligand, *T* tertile

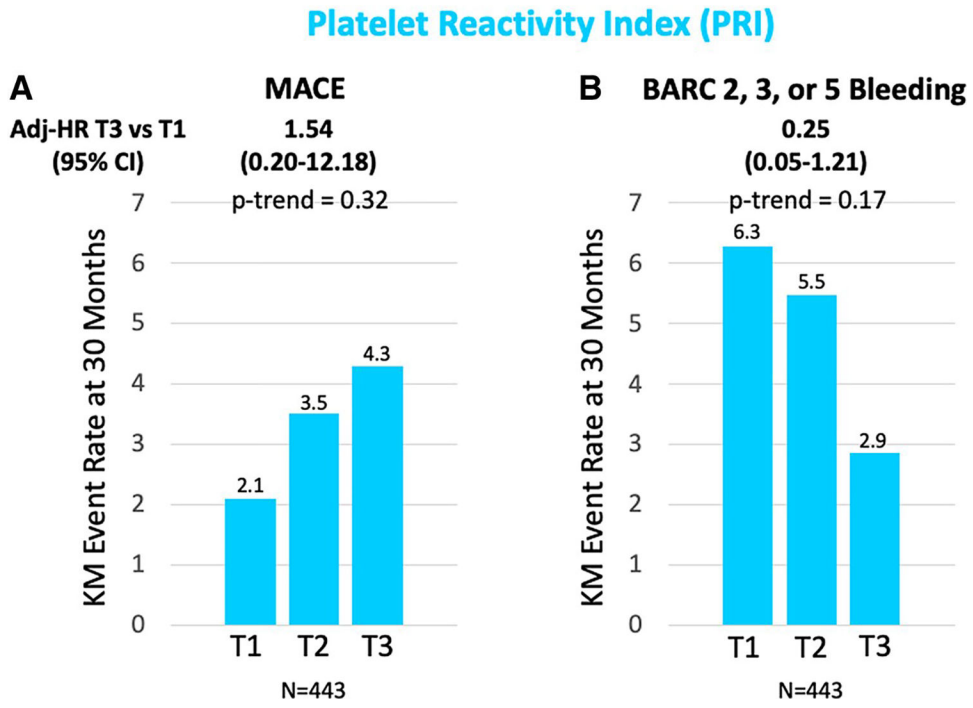

Fig. 2.

Platelet reactivity index (PRI) at 12 months post-PCI and risk of **a** the composite endpoint of cardiovascular death, myocardial infarction, or ischemic stroke and **b** BARC 2, 3, or 5 bleeding through long-term follow-up in patients randomized to continued DAPT. Kaplan–Meier event rates are reported at 18 months post-randomization. *BARC* Bleeding Academic Research Consortium, *DAPT* dual antiplatelet therapy, *KM* Kaplan–Meier, *PRI* platelet reactivity index

Table 1

Aggregate baseline characteristics of patients in the (1) protein biomarkers of platelet activation (n = 1399) and (2) platelet function testing (n = 443) cohorts

Variable	Protein biomarkers cohort (n = 1399)	Platelet function testing cohort (n=443)
Age (years), median (IQR)	63 (55, 69)	63 (56, 70)
Female	27.1% (379)	22.6% (100)
White race	95.1% (1330)	93.9% (416)
BMI, median (IQR)	30.1 (27.0, 33.7)	29.7 (26.8, 33.2)
Diabetes mellitus	30.4% (425)	28.5% (126)
Hypertension	74.0% (1033)	69.3% (307)
Current cigarette smoker or within past year	26.0% (362)	23.4% (103)
Prior stroke/TIA	4.4% (61)	4.3% (19)
Heart failure	4.3% (60)	3.6% (16)
Peripheral arterial disease	6.5% (90)	4.5% (20)
Previous percutaneous coronary intervention (PCI)	32.9% (457)	28.6% (126)
Coronary artery bypass graft (CABG)	13.7% (191)	12.0% (53)
Previous myocardial infarction (MI)	21.4% (292)	19.2% (84)
Indication for index procedure		
STEMI	11.7% (163)	11.3% (50)
NSTEMI	16.0% (224)	15.3% (68)
Unstable angina	14.9% (209)	13.3% (59)
Stable angina	35.4% (495)	36.3% (161)
Other	22.0% (308)	23.7% (105)
Type of stent at index procedure		
DES only	86.2% (1206)	85.6% (379)
BMS only	13.8% (193)	14.4% (64)

BMI body-mass index, *BMS* bare metal stent, *DES* drug-eluting stent, *IQR* interquartile range, *NSTEMI* non-ST-segment elevation myocardial infarction, *STEMI* ST-segment elevation myocardial infarction, *TIA* transient ischemic attack