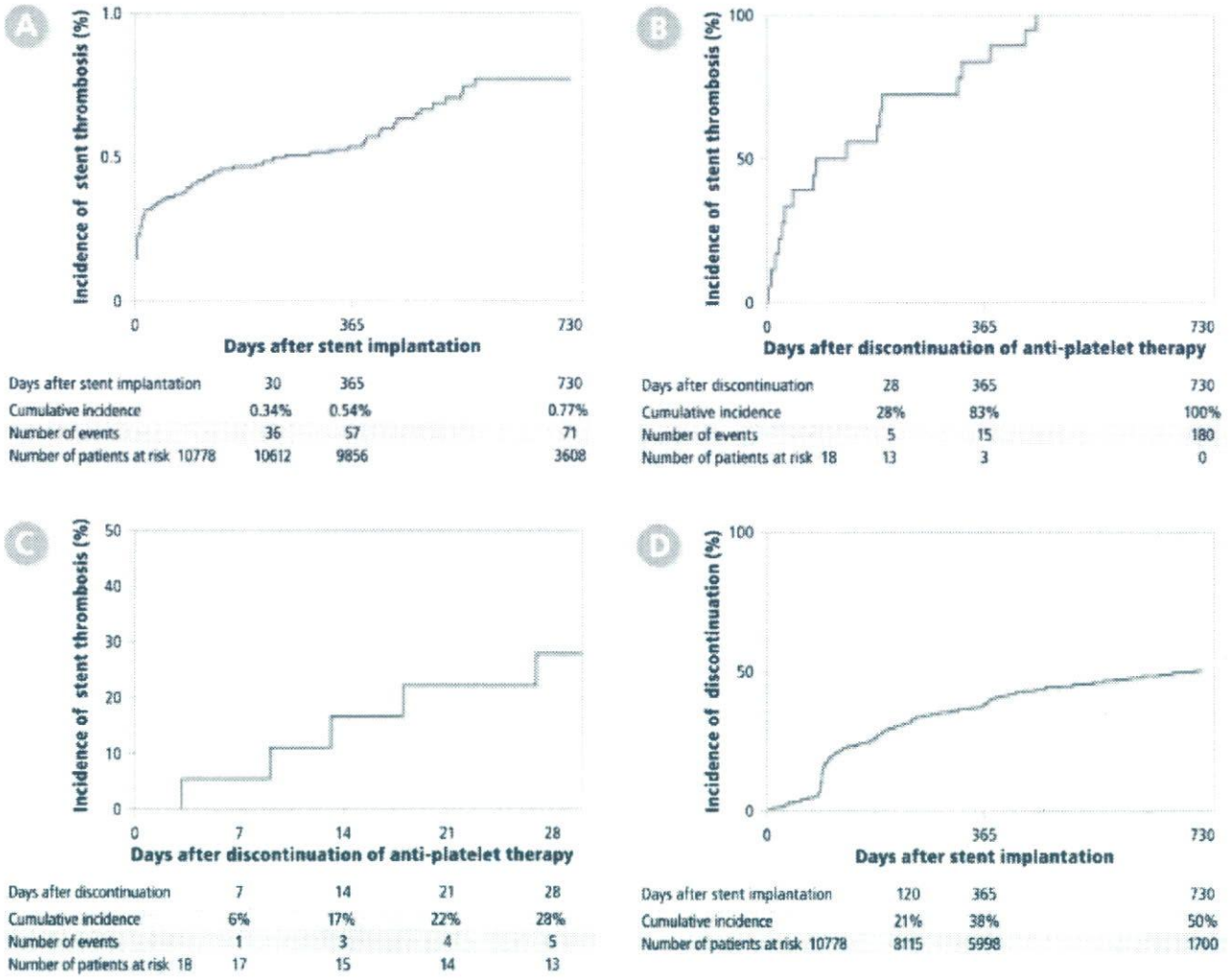


Figure 1. A, Kaplan–Meier curve showing the patient-based cumulative incidence of definite ST. B, Timing of ST after antiplatelet therapy discontinuation. C, Timing of ST within 28 days after antiplatelet therapy discontinuation. D, Kaplan–Meier curve showing the cumulative incidence of persistent thienopyridine discontinuation over time.

graphic measurement. Bifurcation lesion was defined as that involving a side branch of ≥2.2 mm in diameter.

Death was regarded as cardiac in origin unless obvious noncardiac causes could be identified. Any death during the index hospitalization was regarded as cardiac death. Sudden death was defined as unexplained death in previously stable patients. MI was adjudicated according to the definition in the Arterial Revascularization Therapy Study.[6] Within 1 week of the index procedure, only Q-wave MI was adjudicated as MI.

ST was defined according to the Academic Research Consortium definition.[7] Not only sudden death but also those deaths without enough information to exclude sudden death were regarded as possible ST. Unless otherwise noted, definite ST assessed on an individual patient basis was used as the end point for ST, because this was the end point used in a recent large-scale registry for DES.[8]

Antiplatelet Therapy

The recommended antiplatelet regimen was aspirin (≥81 mg daily) indefinitely and thienopyridine (200 mg of ticlopidine or 75 mg of clopidogrel daily) for at least 3 months. Duration of antiplatelet therapy was left to the discretion of each attending physician.

Dates of discontinuation of aspirin and thienopyridine were reported separately on the follow-up forms. When discontinuation was intended to be temporary, the dates the medications were restarted were also reported. When the attending physician intended to discontinue medications permanently, dates related to the restarting of medications after discontinuation were not systematically reported. Persistent discontinuation was defined as withdrawal that lasted at least 2 months.

ST incidences were evaluated according to the status of aspirin therapy and thienopyridine therapy. Analyses were made by time intervals after index PCI (ie, within 30 days, 31 to 180 days, 181 to 365 days, 366 to 548 days, and 549 to 730 days) in accordance with a previous report.[4] Those patients in whom occurrence of ST could be evaluated throughout the given intervals of interest were eligible for the analysis. Patients with known discontinuation of therapy for any duration until the end of the given intervals were assigned to the discontinuation group of patients without ST. In patients with ST, only discontinuation before the onset of ST was evaluated. Patients with acute ST and those with ST during the prior intervals were excluded from the analysis.

The influence of prolonged dual-antiplatelet therapy on clinical outcome was assessed with the so-called landmark analysis reported previously, which is a form of survival analysis that classifies patients on the basis of some nonoutcome event that occurs during follow-up (eg, discontinuation of thienopyridine at 6 months).[9] Eligible patients were those patients who continued taking aspirin and were free from death, MI, stroke, or ST at the 6-month landmark point.

Statistical Analysis

Categorical variables were compared with the χ^2 test. Continuous variables are expressed as mean±SD unless otherwise indicated. Continuous variables were compared with the Student t test or Wilcoxon rank sum test based on the distribution. Cumulative incidence was estimated by the Kaplan–Meier method, and differences were assessed with the log-rank test.

A Cox proportional hazard model was used to identify independent risk factors of ST. We used the variables listed in supplemental Table I as potential independent variables. The continuous variables were dichotomized by clinically meaningful reference values or median values. To determine the independent risk factors, we first selected variables with P values <0.05 in the univariable Cox models and for which proportional hazard assumptions were acceptable on the plots of log (time) versus log [−log (survival)] stratified by the variable. We then included them simultaneously in the multivariable models. Patients with missing values for any selected variable were excluded from the multivariable analysis. The robustness of independent risk factors for ST that were identified by the full model without selection of variables was confirmed by both forward and backward selection procedures.

Landmark analysis was conducted as described previously.[9] We computed the propensity score using logistic regression, with the dependent variable being continued thienopyridine use at 6 months and with the 23 independent variables listed in supplemental Table I. Next, we computed the adjusted survival curves of groups with and without thienopyridine use at the 6-month landmark using the Cox proportional hazard model in conjunction with methods described by Ghali et al,[10] adjusting for the propensity score and the above-mentioned 23 covariates.

All analyses were conducted by a physician (Takeshi Kimura) and an independent statistician (Takeshi Morimoto) with the use of SAS software version 9.1 (SAS Institute Inc, Cary, NC) and S-Plus version 7.0 (Insightful Corp, Seattle, Wash). All reported P values are 2-sided.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Baseline Characteristics

It was common for patients in the present study to have high-risk features, such as age ≥80 years, diabetes mellitus, renal failure, or unprotected left main disease (Table 1). Complex lesions such as chronic total occlusion, long lesions, and small vessels were also common. However, only 21% of patients presented with acute coronary syndrome (ACS). Procedures were characterized by use of high inflation pressure and a high rate of intravascular ultrasound guidance.

Clinical Outcome

Cumulative incidences of death, cardiac death, and sudden death at 2 years were 7.2%, 3.7%, and 1.4%, respectively (Table 2). Incidence of MI was 1.5% at 2 years. MI related to ST constituted 45% of all MIs, or 0.7% at 2 years. Incidence of target-lesion revascularization was 10.2% at 2 years.

Incidence, Clinical Sequelae, and Predictors of ST

Cumulative incidences of ST at 2 years were 0.77% for definite ST, 0.91% for definite or probable ST, and 2.48% for all ST (Table 2). Incidences of definite ST were 0.34% (95% confidence interval [CI] 0.23% to 0.45%) at 30 days, 0.54% (95% CI 0.4% to 0.68%) at 1 year, and 0.77% (95% CI 0.58% to 0.96%) at 2 years (Figure 1A). The slope of the linear portion of the cumulative incidence curve of ST between 30 days and 2 years was 0.2% per year. Clinical sequelae within 30 days of ST were MI in 85% to 95% of cases and death in 11% to 38% of cases, depending on ST timing (Table 3).

Table 3. Clinical Sequelae of ST and Antiplatelet Therapy at the Time of ST

	Early ST (≤30 d) (n=36)	Late ST (31–365 d) (n=21)	Very Late ST (366–730 d) (n=14)
Interim TVR, %	0	9.5	14
Clinical sequelae within 30 days of ST, %			
Death	11	38	18
MI	85	95	91
Q-wave infarction	56	70	83
Non–Q-wave infarction	29	25	8
Emergency CABG	11	0	0
Antiplatelet therapy at time of ST, %			
On dual antiplatelet therapy	86	57	36
On aspirin alone	8.3	14	43
On thienopyridine alone	0	4.8	0
Off both	2.8	24	21
Unknown	2.8	0	0
Discontinuation of thienopyridine, n	1	8	9
Median interval (IQR) between thienopyridine discontinuation and ST, d	9	29 (14–174)	196 (82–404)

TVR indicates target-vessel revascularization; IQR, interquartile range.

Univariable predictors for ST are shown in supplemental Table I. Multivariable analysis identified ACS (hazard ratio [HR] 2.53, 95% CI 1.3 to 4.92, P=0.006) and heart failure (HR 2.33, 95% CI 1.12 to 4.84, P=0.02) as independent predictors of early ST. Independent predictors of late or very late ST included hemodialysis (HR 6.86, 95% CI 3.05 to 15.45, P<0.001), end-stage renal disease (estimated glomerular filtration rate <30 mL · min^{-1} · 1.73 m^{-2}) without hemodialysis (HR 5.33, 95% CI 2.0 to 14.15, P<0.001), side-branch stenting (HR 3.5, 95% CI 1.36 to 9.03, P=0.01), and smoking (HR 2.36, 95% CI 1.17 to 4.76, P=0.02).

Discontinuation of Antiplatelet Therapy and ST

During the index hospitalization, aspirin and thienopyridine were administered in 98.9% and 99.5% (ticlopidine 96.9% and clopidogrel 2.6%) of patients, respectively. Cilostazol was administered in 3.2% of patients at the time of hospital discharge.

The status of antiplatelet therapy immediately before the onset of ST was known for the vast majority of patients with ST, except for 1 patient who presented with cardiogenic shock. The majority of patients (86%) with early ST were taking dual-antiplatelet therapy at the time of ST. The prevalence of dual therapy was 57% for late ST and 36% for very late ST, respectively (Table 3). Among 18 patients who

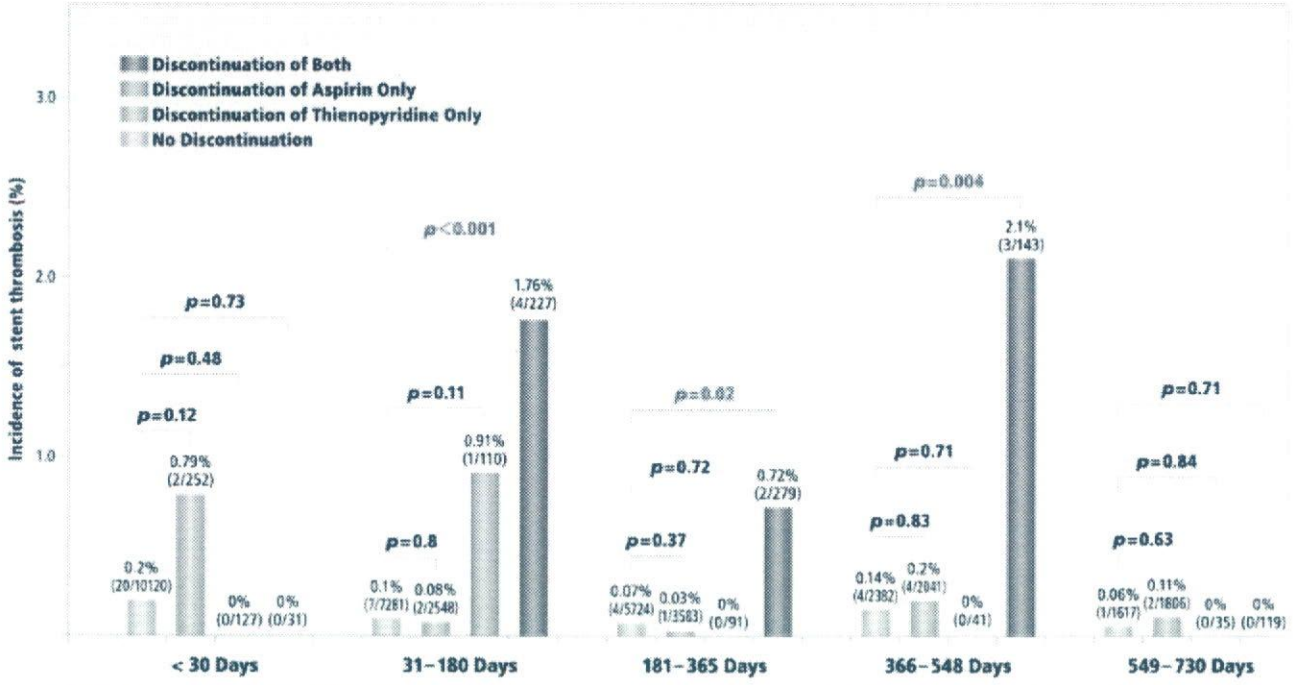


Figure 2. Relationship between thienopyridine and/or aspirin discontinuation and ST by time interval after stent implantation.

had ST after any antiplatelet therapy discontinuation, the majority of ST events occurred >1 week after discontinuation (Figure 1B and 1C).

Thienopyridine use was maintained in 97%, 62%, and 50% of patients at 30 days, 1 year, and 2 years, respectively. A steep rise was found at ≈3 months in the cumulative incidence curve of persistent discontinuation of thienopyridine (Figure 1D); however, a corresponding steep rise was not observed in the cumulative ST incidence curve (Figure 1A).

With regard to the relation between aspirin and/or thienopyridine discontinuation and ST, patients who discontinued both aspirin and thienopyridine had a significantly higher ST rate than those who continued both agents in the intervals of 31 to 180 days, 181 to 365 days, and 366 to 548 days after stent implantation (1.76% versus 0.1%, $P<0.001$; 0.72% versus 0.07%, $P=0.02$; and 2.1% versus 0.14%, $P=0.004$, respectively; Figure 2). When discontinuation of aspirin was considered, discontinuation of thienopyridine therapy only was not associated with increased ST risk in any of the time intervals.

Landmark Analysis Based on Thienopyridine Use

At 6 months, thienopyridine use was maintained in 7247 (73%) of 9875 patients eligible for the landmark analysis. Patients taking thienopyridine had significantly more complex characteristics, although some of these statistically significant differences might not be clinically relevant (Table 4). After adjustment for differences in baseline characteristics, the rates of death, MI, death or MI, and a combined end point of cardiac death, MI, or stroke at 24 months were not different between the 2 groups with or without thienopyridine therapy in the 6-month landmark analysis (Figure 3; Table 5).

The influence of ACS on the 6-month landmark analysis was evaluated. The cumulative rate of death or MI was significantly higher in patients with ACS than in those without ACS (Figure 4A); however, rates of death or MI beyond 6 months were similar in both groups (Figure 4B). Adjusted rates of death or MI at 24 months in the 2 groups either taking or not taking thienopyridine therapy at 6 months were similar in patients with or without ACS (Figure 4C and 4D).

Discussion

The main findings of the present study are that discontinuation of both aspirin and thienopyridine, but not discontinuation of thienopyridine therapy only, is associated with an increased ST risk and that no apparent clinical benefit is received from thienopyridine use beyond 6 months after SES implantation. More than 2 years ago, concerns were raised about DES safety.[1,2] Although more recent reports from registries and meta-analyses of randomized, controlled trials provided data supporting the relative safety of DES compared with BMS,[11,12] a cohort study conducted in Bern, Switzerland, and Rotterdam, Netherlands, demonstrated that definite ST continues to occur at the constant rate of 0.6% per year from 30 days to 3 years after DES implantation.[8] The present ongoing analysis using the same ST end point also showed that ST remained a continuous hazard up to 2 years after SES implantation, although the cumulative incidence of ST appeared to be considerably lower than that in the Bern and Rotterdam cohorts.[8]

We can suggest several potential reasons for the markedly lower rate of early ST in the present registry. First, there might be ethnic differences in the propensity for ST. We reported a 0.9% rate of early ST in 320 Japanese patients undergoing planned BMS implantation with an antithrom-

Table 4. Baseline and Procedural Characteristics in the 2 Groups Analyzed by Landmark Analysis at 6 Months

	Taking Thienopyridine (n=7247)	Not Taking Thienopyridine (n=2628)	*P*
Age, y	68.1±10.2	68.3±10.0	0.44
Male, %	76	75	0.12
Body mass index, kg/m^2	24.0±3.4	24.0±3.4	0.98
Hypertension, %	76	74	0.03
Diabetes mellitus, %	41	39	0.2
Current smoking, %	19	21	0.11
eGFR <30 mL · min^{-1} · 1.73 m^{-2}, %	9.7	8.0	0.009
ACS, %	20	22	0.03
Prior MI, %	28	28	0.62
Prior stroke, %	9.3	8.1	0.06
Peripheral vascular disease, %	11	12	0.54
Prior heart failure, %	13	10	<0.001
Prior PCI, %	50	44	<0.001
Prior CABG, %	7.3	7.0	0.58
Multivessel stenting, %	21	16	0.14
Target of unprotected LMCA, %	4.1	2.6	<0.001
Target of proximal LAD, %	49	49	0.51
Target of chronic total occlusion, %	12	9.7	0.005
Target of in-stent restenosis, %	17	15	0.046
Target lesion <2.5 mm in diameter, %	33	33	0.93
Intravascular ultrasound use, %	49	44	<0.001
Side-branch stenting, %	4.9	2.5	<0.001
Total length of stents, mm	39.8±26.5	36.0±22.5	<0.001

eGFR indicates estimated glomerular filtration rate; LMCA, left main coronary artery; and LAD, left anterior descending coronary artery.

Continuous variables were expressed as mean±SD.

botic regimen that included aspirin and warfarin.[13] This early ST rate appears to be markedly lower than the 2.7% in 550 US patients reported in the Stent Anticoagulation Restenosis Study using the same antithrombotic regimen and the same BMS.[14] Second, the incidence of ACS presentation, which is an established risk factor for early ST, was lower in the present study population than in the Bern and Rotterdam cohorts[9] (21% and 59%, respectively). Third, only 3% of patients in the present study discontinued thienopyridine within 30 days of SES implantation compared with the 14% discontinuation rate reported in the PREMIER registry (Prospective Registry Evaluating Myocardial Infarction: Events and Recovery).[5] We cannot provide a clear explanation for the lower rate of late and very late ST in the present study population compared with that in the Bern and Rotterdam cohorts,[8] because the mechanisms of late and very late ST have not yet been well clarified.

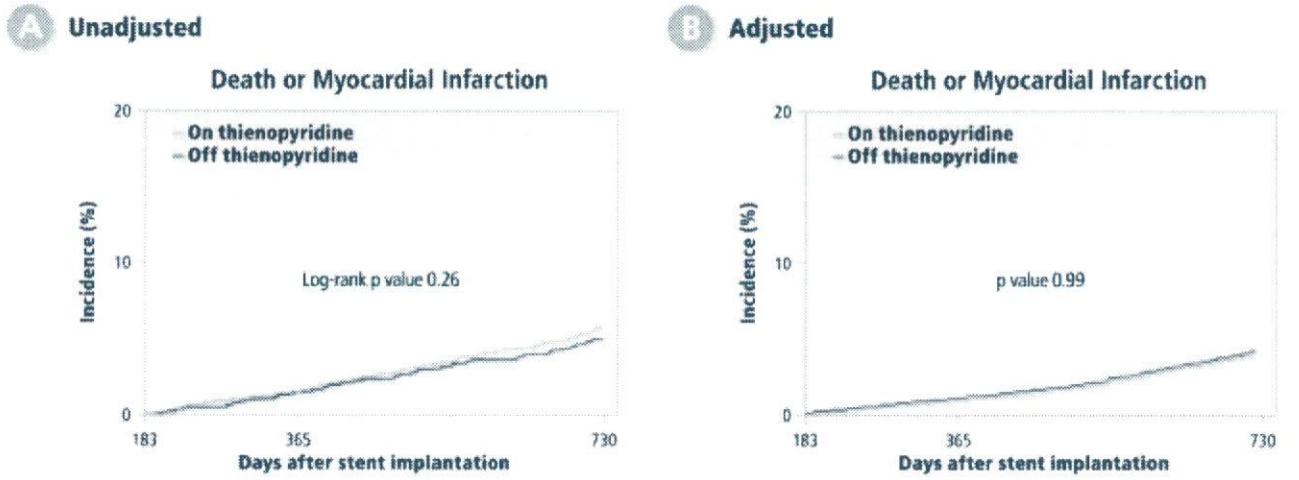
Although no randomized study has evaluated the role of dual-antiplatelet therapy in DES, the benefit of dual-antiplatelet therapy in preventing ST within 1 month after BMS implantation has been well established from randomized trials.[14,15] The TRITON-TIMI 38 trial (Trial to Assess Improvements in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis In Myocardial Infarction) also demonstrated that more intensive antiplatelet therapy with prasugrel was associated with a marked reduction in ST rate compared with standard antiplatelet therapy with clopidogrel.[16] Reduction of the ST rate in the prasugrel group was predominantly seen within 30 days after stent implantation, although the reduction in the rate of late ST was also of borderline significance. Therefore, the role of intensive antiplatelet therapy in reducing early ST appears to be firmly established.

However, the role of thienopyridine therapy in reducing late ST beyond 1 month after stent implantation has not been well addressed. Although premature discontinuation of antiplatelet therapy has been reported to be the most powerful predictor of ST and/or adverse outcome,[3–5] these previous reports did not discriminate the relative impact of discontinuation of either aspirin, thienopyridine, or both agents. The present analysis demonstrated that withdrawal of both thienopyridine and aspirin, but not of thienopyridine therapy alone, was associated with increased ST risk beyond 1 month after SES implantation. Aspirin withdrawal was reported to be responsible for admission with an ACS in 51 (4%) of 1236 patients, with a mean delay between aspirin cessation and hospitalization of 10±2 days.[17] It is noteworthy that late and very late ST at a mean of 16±7 months after BMS implantation was responsible for ACS in 10 (20%) of these 51 patients.

Furthermore, only one third of ST events after discontinuation of antiplatelet therapy (mostly thienopyridine) occurred within the first 28 days after discontinuation. This might lead to a discussion about whether or not a direct link in fact exists between discontinuation of antiplatelet therapy and ST, particularly very late ST.

Previous prospective studies demonstrated a clinical benefit of the prolonged use of thienopyridine for up to 1 year in patients undergoing PCI with BMS,[18,19] primarily in the setting of ACS. Extrapolation of these findings to DES might make it appear reasonable to advocate adherence to dual-antiplatelet therapy for 1 year after DES implantation; however, the present study results suggest that it is also reasonable to discontinue thienopyridine and adhere to aspirin monotherapy in situations in which continuation of dual-antiplatelet therapy appears to be otherwise clinically irrelevant. Furthermore, because the majority of ST events occurred >1 week after discontinuation of antiplatelet therapy, it appears important to make the duration of discontinuation as short as possible if discontinuation is unavoidable.

The optimal duration of dual-antiplatelet therapy has not been well established. A single-center observational study of 1216 DES patients and 2393 BMS patients reported that use of clopidogrel at 6 and 12 months was associated with a lower incidence of death or MI at 24 months in patients with DES but not in patients with BMS.[9] On the other hand, a similar



Days after stent implantation	183	365	730
Cumulative incidence, On thienopyridine		1.6%	5.8%
Number of events		115	268
Number of patients at risk	7247	6918	2405
Cumulative incidence, Off thienopyridine		1.5%	5.0%
Number of events		39	91
Number of patients at risk	2628	2533	1072

Days after stent implantation	183	365	730
Cumulative incidence, On thienopyridine		1.1%	4.1%
Cumulative incidence, Off thienopyridine		1.3%	4.1%

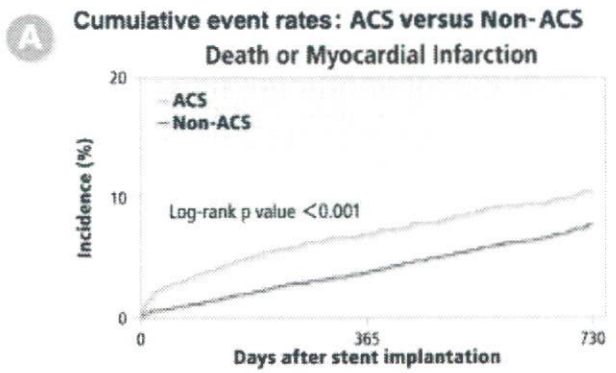
Figure 3. Unadjusted and adjusted cumulative incidences of death or MI using the 6-month landmark analysis.

analysis in 671 diabetic patients reported that use of clopidogrel at 6 months was associated with a significantly lower incidence of death or MI at 18 months in patients with BMS but not in those with DES.[20] Relatively small sample sizes in these subgroup analyses to evaluate hard clinical end points might be 1 of the reasons for the discrepancy. The present analysis of a larger number of patients by the same method demonstrated a similar long-term clinical outcome regardless of thienopyridine use at 6 months in SES patients. Given the increased risk of bleeding and the huge economic burden with prolonged dual-antiplatelet therapy,[18,19,21] the optimal duration of dual-antiplatelet therapy should be defined by prospective randomized trials evaluating the net clinical benefit after considering both ischemic events and bleeding complications.

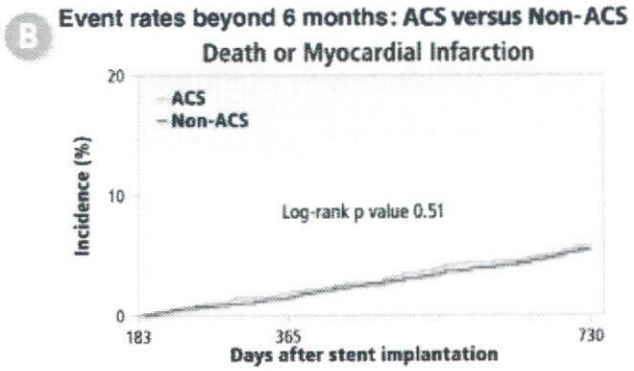
The present study has several important limitations. First, baseline characteristics and procedural characteristics such as the high rate of intravascular ultrasound guidance in the present cohort might be markedly different from practices outside Japan. Also, ticlopidine was used as a thienopyridine antiplatelet agent in the vast majority of patients, in contrast to the use of clopidogrel in most other studies. These and other ethnic differences might make it difficult to apply the findings in the present study outside of Japan. Second, although data on antiplatelet therapy use were collected prospectively, no attempt was made to verify compliance with antiplatelet medications for patients in whom discontinuation was not reported; this might well have led to overestimation of compliance. In addition, we did not systematically evaluate the restarting of antiplatelet therapy after persistent discontinuation, which could have resulted in the potential underestimation of medication use. In fact, in the 6-month landmark analysis, 13% of patients who underwent repeated revascularization >6 months after the first procedure were likely to have restarted thienopyridine. The limitation of a landmark analysis is that it only examines specific points in time. A Cox proportional hazards model with a time-dependent covariate (thienopyridine discontinuation) might be able to examine the continuous risk of thienopyridine discontinuation. Furthermore, when follow-up information was obtained by contact with patients, dates of discontinuation of aspirin and thienopyridine were based on retrospective recall by the patients or relatives, which suggests a potential for recall bias. Third, unmeasured confounders related to thienopyridine discontinuation might be present because of the observational study design. Fourth, the number of patients at risk at 2-year follow-up was limited. Therefore, the results of the present study might be valid only during the first year after SES implantation. Finally, bleeding complications were not evaluated, which made it impossible to evaluate the net clinical efficacy of dual-antiplatelet therapy.

Table 5. Unadjusted and Adjusted 24-Month Outcomes Based on 6-Month Thienopyridine Use

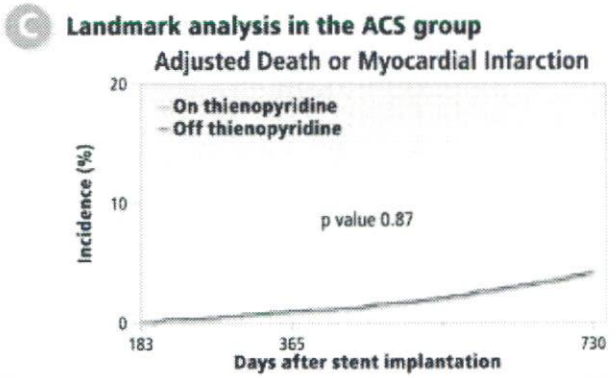
	No. of Events					Unadjusted Event Rates, %				Adjusted Event Rates, %			
	No. at Risk at 6 Months	Death	MI	Death or MI	Cardiac Death, MI, or Stroke	No. at Risk at 24 Months	Death (P=0.22)	MI (P=0.81)	Death or MI (P=0.26)	Cardiac Death, MI, or Stroke (P=0.6)	Death (P=0.9)	MI (P=0.42)	Death or MI (P=0.99)	Cardiac Death, MI, or Stroke (P=0.79)
On thienopyridine	7247	238	41	268	228	2424	5.2	0.9	5.8	4.9	3.4	0.6	4.1	4.0
Off thienopyridine	2628	79	17	91	82	1078	4.4	0.8	5.0	4.3	3.4	0.8	4.1	4.1



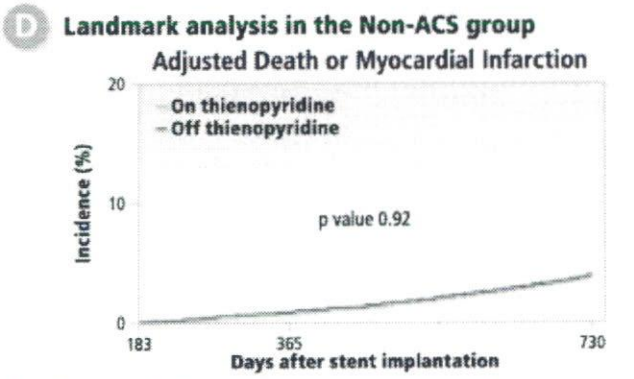
Days after stent implantation	0	183	365	730
Cumulative incidence, ACS		5.1%	6.9%	10.4%
Number of events		116	157	194
Number of patients at risk	2308	2132	2018	650
Cumulative incidence, Non-ACS		2.1%	3.7%	7.7%
Number of events		179	307	483
Number of patients at risk	8470	8162	7816	2932



Days after stent implantation	183	365	730
Cumulative incidence, ACS		1.9%	5.7%
Number of events		38	75
Number of patients at risk	2025	1919	627
Cumulative incidence, Non-ACS		1.5%	5.5%
Number of events		116	284
Number of patients at risk	7850	7532	2850



Days after stent implantation	183	365	730
Cumulative incidence, On thienopyridine		1.3%	4.2%
Number of events		31	55
Number of patients at risk	1447	1364	438
Cumulative incidence, Off thienopyridine		1.3%	4.1%
Number of events		7	20



Days after stent implantation	183	365	730
Cumulative incidence, On thienopyridine		1.1%	3.9%
Number of events		84	213
Number of patients at risk	5800	5554	1967
Cumulative incidence, Off thienopyridine		1.1%	4.0%
Number of events		32	71

Figure 4. A, Cumulative rates of death or MI in patients with or without ACS. B, Rates of death or MI beyond 6 months in patients with or without ACS. C and D, Adjusted cumulative incidences of death or MI using the 6-month landmark analysis in patients with and without ACS.

Despite these limitations, we would conclude that discontinuation of both thienopyridine and aspirin, but not discontinuation of thienopyridine therapy alone, was associated with an increased risk of ST. Landmark analysis did not suggest an apparent clinical benefit of thienopyridine use beyond 6 months after SES implantation.

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Disclosures

Dr Kimura serves as an advisory board member and member of the speakers' bureau for Cordis Cardiology and has received honoraria from Cordis Cardiology. Dr Nakagawa is a member of the speakers' bureau and has received honoraria from Cordis Cardiology. Dr Miyazaki is an advisory board member and receives honoraria from Cordis Cardiology. Drs Shiode and Mitsudo both report receipt of honoraria from Cordis Cardiology. The remaining authors report no conflicts.

References

1. McFadden EP, Stabile E, Regar E, Cheneau E, Ong AT, Kinnaird T, Suddath WO, Weissman NJ, Torguson R, Kent KM, Pichard AD, Satler LF, Waksman R, Serruys PW. Late thrombosis in drug-eluting coronary stents after discontinuation of antiplatelet therapy. *Lancet*. 2004;364:1519–1521.
2. Lagerqvist B, James SK, Stenestrand U, Lindbäck J, Nilsson T, Wallentin L; SCAAR Study Group. Long-term outcomes with drug-eluting stents versus bare-metal stents in Sweden. *N Engl J Med*. 2007;356:1009–1019.
3. Iakovou I, Schmidt T, Bonizzoni E, Ge L, Sangiorgi GM, Stankovic G, Airoldi F, Chieffo A, Montorfano M, Carlino M, Michev I, Corvaja N, Briguori C, Gerckens U, Grube E, Colombo A. Incidence, predictors, and outcome of thrombosis after successful implantation of drug-eluting stents. *JAMA*. 2005;293:2126–2130.
4. Airoldi F, Colombo A, Morici N, Latib A, Cosgrave J, Buellesfeld L, Bonizzoni E, Carlino M, Gerckens U, Godino C, Melzi G, Michev I, Montorfano M, Sangiorgi GM, Qasim A, Chieffo A, Briguori C, Grube E. Incidence and predictors of drug-eluting stent thrombosis during and after discontinuation of thienopyridine treatment. *Circulation*. 2007;116:745–754.

5. Spertus JA, Kettelkamp R, Vance C, Decker C, Jones PG, Rumsfeld JS, Messenger JC, Khanal S, Peterson ED, Bach RG, Krumholz HM, Cohen DJ. Prevalence, predictors, and outcomes of premature discontinuation of thienopyridine therapy after drug-eluting stent placement: results from the PREMIER registry. *Circulation*. 2006;113:2803–2809.
6. Serruys PW, Ong AT, van Herwerden LA, Sousa JE, Jatene A, Bonnier JJ, Schönberger JP, Buller N, Bonser R, Disco C, Backx B, Hugenholtz PG, Firth BG, Unger F. Five-year outcomes after coronary stenting versus bypass surgery for the treatment of multivessel disease: the final analysis of the Arterial Revascularization Therapies Study (ARTS) randomized trial. *J Am Coll Cardiol*. 2005;46:575–581.
7. Mauri L, Hsieh WH, Massaro JM, Ho KK, D'Agostino R, Cutlip DE. Stent thrombosis in randomized clinical trials of drug-eluting stents. *N Engl J Med*. 2007;356:1020–1029.
8. Daemen J, Wenaweser P, Tsuchida K, Abrecht L, Vaina S, Morger C, Kukreja N, Jüni P, Sianos G, Hellige G, van Domburg RT, Hess OM, Boersma E, Meier B, Windecker S, Serruys PW. Early and late coronary stent thrombosis of sirolimus-eluting and paclitaxel-eluting stents in routine clinical practice: data from a large two-institutional cohort study. *Lancet*. 2007;369:667–678.
9. Eisenstein EL, Anstrom KJ, Kong DF, Shaw LK, Tuttle RH, Mark DB, Kramer JM, Harrington RA, Matchar DB, Kandzari DE, Peterson ED, Schulman KA, Califf RM. Clopidogrel use and long-term clinical outcomes after drug-eluting stent implantation. *JAMA*. 2007;297:159–168.
10. Ghali WA, Quan H, Brant R, van Melle G, Norris CM, Faris PD, Galbraith PD, Knudtson ML; APPROACH (Alberta Provincial Project for Outcome Assessment in Coronary Heart Disease) Investigators. Comparison of 2 methods for calculating adjusted survival curves from proportional hazards models. *JAMA*. 2001;286:1494–1497.
11. Tu JV, Bowen J, Chiu M, Ko DT, Austin PC, He Y, Hopkins R, Tarride JE, Blackhouse G, Lazzam C, Cohen EA, Goeree R. Effectiveness and safety of drug-eluting stents in Ontario. *N Engl J Med*. 2007;357:1393–1402.
12. Kastrati A, Mehilli J, Pache J, Kaiser C, Valgimigli M, Kelbaek H, Menichelli M, Sabaté M, Suttorp MJ, Baumgart D, Seyfarth M, Pfisterer ME, Schömig A. Analysis of 14 trials comparing sirolimus-eluting stents with bare-metal stents. *N Engl J Med*. 2007;356:1030–1039.
13. Kimura T, Tamura T, Yokoi H, Nobuyoshi M. Long-term clinical and angiographic follow-up after placement of Palmaz-Schatz coronary stent: a single center experience. *J Interv Cardiol*. 1994;7:129–139.
14. Leon MB, Baim DS, Popma JJ, Gordon PC, Cutlip DE, Ho KK, Giambartolomei A, Diver DJ, Lasorda DM, Williams DO, Pocock SJ, Kuntz RE; Stent Anticoagulation Restenosis Study Investigators. A clinical trial comparing three antithrombotic-drug regimens after coronary-artery stenting. *N Engl J Med*. 1998;339:1665–1671.
15. Schömig A, Neumann FJ, Kastrati A, Schühlen H, Blasini R, Hadamitzky M, Walter H, Zitzmann-Roth EM, Richardt G, Alt E, Schmitt C, Ulm K. A randomized comparison of antiplatelet and anticoagulant therapy after the placement of coronary-artery stents. *N Engl J Med*. 1996;334:1084–1089.
16. Wiviott SD, Braunwald E, McCabe CH, Horvath I, Keltai M, Herrman JP, Van de Werf F, Downey WE, Scirica BM, Murphy SA, Antman EM; TRITON-TIMI 38 Investigators. Intensive oral antiplatelet therapy for reduction of ischaemic events including stent thrombosis in patients with acute coronary syndromes treated with percutaneous coronary intervention and stenting in the TRITON-TIMI 38 trial: a subanalysis of a randomised trial. *Lancet*. 2008;371:1353–1363.
17. Ferrari E, Benhamou M, Cerboni P, Marcel B. Coronary syndromes following aspirin withdrawal: a special risk for late stent thrombosis. *J Am Coll Cardiol*. 2005;45:456–459.
18. Mehta SR, Yusuf S, Peters RJ, Bertrand ME, Lewis BS, Natarajan MK, Malmberg K, Rupprecht H, Zhao F, Chrolavicius S, Copland I, Fox KA; Clopidogrel in Unstable angina to prevent Recurrent Events trial (CURE) Investigators. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. *Lancet*. 2001;358:527–533.
19. Steinhubl SR, Berger PB, Mann JT III, Fry ET, DeLago A, Wilmer C, Topol EJ; CREDO Investigators. Clopidogrel for the Reduction of Events During Observation. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. *JAMA*. 2002;288:2411–2420.
20. Brar SS, Kim J, Brar SK, Zadegan R, Ree M, Liu IL, Mansukhani P, Aharonian V, Hyett R, Shen AY. Long-term outcomes by clopidogrel duration and stent type in a diabetic population with de novo coronary artery lesions. *J Am Coll Cardiol*. 2008;51:2220–2227.
21. Bhatt DL, Fox KA, Hacke W, Berger PB, Black HR, Boden WE, Cacoub P, Cohen EA, Creager MA, Easton JD, Flather MD, Haffner SM, Hamm CW, Hankey GJ, Johnston SC, Mak KH, Mas JL, Montalescot G, Pearson TA, Steg PG, Steinhubl SR, Weber MA, Brennan DM, Fabry-Ribaudo L, Booth J, Topol EJ; CHARISMA Investigators. Clopidogrel and aspirin versus aspirin alone for the prevention of atherothrombotic events. *N Engl J Med*. 2006;354:1706–1717.

CLINICAL PERSPECTIVE

Randomized data are lacking on the optimal duration of dual-antiplatelet therapy after drug-eluting stent implantation and on the risks associated with discontinuation of dual-antiplatelet therapy. Despite the absence of randomized data, the use of dual-antiplatelet therapy beyond 1 year has become commonplace in clinical practice. In the j-Cypher registry, 10 778 Japanese patients treated exclusively by sirolimus-eluting stents were followed up for up to 2 years with prospective data collection on the status of antiplatelet therapy during follow-up. Incidences of definite stent thrombosis were 0.34% at 30 days, 0.54% at 1 year, and 0.77% at 2 years. Thienopyridine use was maintained in 97%, 62%, and 50% of patients at 30 days, 1 year, and 2 years, respectively. The main findings of the present study were that discontinuation of both aspirin and thienopyridine, but not discontinuation of thienopyridine therapy only, was associated with an increased stent thrombosis risk and that no apparent clinical benefit of thienopyridine use could be seen beyond 6 months after sirolimus-eluting stent implantation, according to the 6-month landmark analysis. Given the increased risk of bleeding and huge economic burden associated with prolonged dual-antiplatelet therapy, the optimal duration of dual-antiplatelet therapy should be defined by prospective randomized trials evaluating its net clinical benefit after consideration of both ischemic events and bleeding complications.

SUPPLEMENTAL MATERIAL.

Supplemental table 1. Univariable Predictors of Early and Late or Very Late Stent Thrombosis

A. Early stent thrombosis

Variables	Yes		No		
	N of patients	Event rate	N of patients	Event rate	p Value
Age ≥80 years	1362	0.22%	9416	0.35%	0.44
Male gender	8123	0.4%	2655	0.15%	0.06
Body mass index < 25.0	7004	0.27%	3771	0.45%	0.12
Hypertension	8069	0.34%	2709	0.33%	0.99
Diabetes mellitus	4400	0.39%	6378	0.3%	0.43
Current smoking	2119	0.52%	8659	0.29%	0.1
e GFR < 30 ml/min/1.73 ㎡					
without hemodialysis	522	0%	9659	0.34%	0.19
with hemodialysis	594	0.52%	10184	0.32%	0.45
Acute coronary syndrome	2308	0.65%	8470	0.25%	0.003
Prior myocardial infarction	3024	0.33%	7754	0.34%	0.97

Prior stroke	1007	0.6%	9771	0.31%	0.13
Peripheral vascular disease	1276	0.24%	9502	0.35%	0.51
Prior heart failure	1460	0.7%	9318	0.28%	0.01
Prior PCI	5179	0.29%	5599	0.38%	0.44
Prior CABG	787	0.26%	9991	0.34%	0.69
Multi-vessel stenting	2089	0.38%	8689	0.32%	0.67
Target of proximal LAD	5284	0.46%	5494	0.22%	0.03
Target of unprotected LMCA	419	0.25%	10359	0.34%	0.73
Target of in-stent restenosis	1705	0.24%	9071	0.35%	0.44
Target of chronic total occlusion	1210	0.25%	9568	0.35%	0.58
Side-branch stenting	465	0.65%	10313	0.32%	0.23
Total stent length > 28 mm	5339	0.4%	5434	0.28%	0.29
Reference diameter pre < 2.5 mm	3582	0.28%	7053	0.37%	0.45
Use of intravascular ultrasound	5152	0.43%	5599	0.25%	0.11

B. Late or very late stent thrombosis

Variables	Yes		No		
	N of patients	Event rate	N of patients	Event rate	p Value

Age ≥80 years	1359	0.35%	9383	0.45%	0.26
Male gender	8091	0.45%	2651	0.39%	0.83
Body mass index < 25.0	6985	0.54%	3754	0.26%	0.054
Hypertension	8042	0.45%	2700	0.4%	0.97
Diabetes mellitus	4383	0.47%	6359	0.42%	0.19
Current smoking	2108	0.84%	8634	0.34%	0.03
e GFR < 30 ml/min/1.73 m^2					
without hemodialysis	522	1.34%	9626	0.33%	<0.001
with hemodialysis	591	1.69%	10151	0.37%	<0.001
Acute coronary syndrome	2293	0.54%	8449	0.42%	0.69
Prior myocardial infarction	3014	0.57%	7728	0.39%	0.43
Prior stroke	1001	0.21%	9741	0.46%	0.5
Peripheral vascular disease	1273	0.25%	9469	0.46%	0.57
Prior heart failure	1450	0.51%	9292	0.43%	0.39
Prior PCI	5164	0.42%	5578	0.46%	0.93
Prior CABG	785	0.66%	9957	0.42%	0.12
Multi-vessel stenting	2081	0.56%	8661	0.41%	0.6
Target of proximal LAD	5260	0.4%	5482	0.47%	0.49

Target of unprotected LMCA	418	0.78%	10324	0.43%	0.14
Target of in-stent restenosis	1701	0.51%	9039	0.43%	0.63
Target of chronic total occlusion	1207	0.41%	9535	0.44%	0.98
Side-branch stenting	462	1.14%	10280	0.41%	0.004
Total stent length > 28 mm	5318	0.58%	5419	0.3%	0.12
Reference diameter pre < 2.5 mm	3572	0.63%	7027	0.36%	0.11
Use of intravascular ultrasound	5130	0.48%	5585	0.4%	0.66

CABG = coronary artery bypass grafting, e GFR = estimated glomerular filtration rate, LAD = left anterior descending coronary artery, LMCA = left main coronary artery and PCI = percutaneous coronary intervention

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Clopidogrel Resistance in Japanese Patients Scheduled for Percutaneous Coronary Intervention

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Background Dual antiplatelet therapy with acetylsalicylic acid (ASA) and a P2Y12 ADP-receptor blocker is standard for prevention of coronary stent thrombosis. Clopidogrel, a 2nd-generation P2Y12 blocker, has recently become available in Japan and this study aimed to evaluate its antiplatelet effects in Japanese patients.

Methods and Results Thirty Japanese patients scheduled for elective coronary stent implantation were enrolled. Under low-dose ASA therapy, 300 mg clopidogrel was loaded on the 1st day and a daily 75-mg dose was administered on the following days. Assessed by optical aggregometer, rapid inhibition occurred at 4 h, when the inhibition of platelet aggregation rate (IPA) was 16.4±12.8% using 5 μmol/L ADP as the stimulus. The antiplatelet efficacy of clopidogrel was reasonably constant in each patient throughout the study period, although there was a broad inter-individual variation. At 48 h after clopidogrel loading, the ratios of responders (IPA ≥30%), hypo-responders (10%≤IPA<30%), and non-responders (IPA <10%) were 36%, 50%, and 14%, respectively.

Conclusions The antiplatelet effectiveness of clopidogrel appeared individual-specific with wide inter-individual variation. The rate of clopidogrel non-responders was 14% among the examined Japanese patients. (*Circ J* 2009; **73:** 336–342)

Key Words: Adenosine diphosphate; Antiplatelet drug; Clopidogrel; Coronary stent; Thienopiridine

Percutaneous coronary intervention (PCI) with coronary stent implantation is performed worldwide for ischemic heart disease. In Japan, 153,501 patients underwent this therapy in 2006, as described in the surveillance report from the Japan Circulation Society. One of the most serious problems is acute and late thrombosis at the site of stenting and much effort had been made to avoid this critical complication. The current standard dual antiplatelet therapy with acetylsalicylic acid (ASA) and thienopyridine ADP-receptor blocker has proven to be a powerful preventive solution[1–5]

Two thienopyridine antiplatelet agents are currently available: ticlopidine and clopidogrel. Although ticlopidine, a 1st-generation thienopyridine, has contributed much to the prevention of stent thrombosis, it frequently causes adverse side-effects such as agranulocytosis, thrombotic thrombocytopenic purpura and liver injury. Clopidogrel, a 2nd-generation P2Y12 blocker, has a better safety profile with a lower incidence of hematologic and liver complications, and has now largely replaced ticlopidine in clinical practice.

One important problem with clopidogrel is the wide inter-individual variation in its antiplatelet effect[3,6–8] It has been demonstrated that clopidogrel does not exert an antiplatelet effect in a certain proportion of patients in Western populations[9] known as clopidogrel resistance. Importantly, several studies have revealed that cardiovascular risk is elevated in patients with clopidogrel resistance[10]

On the other hand, there are well-established differences in the atherothrombotic and hemorrhagic risks in the Japanese compared with Western populations[11] so results from clinical trials in the West using novel antithrombotic agents cannot be applied directly to Japanese patients. Furthermore, the standard dose of ticlopidine for the Japanese (200 mg/day) is much lower than that for Western people (500 mg/day), but the same daily maintenance dose (75 mg) of clopidogrel is used in both populations. Therefore, some Japanese physicians are concerned about the strength of the effect of clopidogrel in Japanese patients and because of those concerns, we designed the present study to evaluate the antiplatelet effects of clopidogrel under low-dose ASA therapy in 30 Japanese patients scheduled for PCI.

Methods

Study Protocol

This study was approved by the Ethics Committee of Kyoto University Hospital, and written informed consent was given by all enrolled patients, who were undergoing elective

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coronary stent implantation. The initial diagnosis of ischemic heart disease was based on symptoms, a non-invasive examination such as stress electrocardiogram, and/or coronary computed tomography angiography. Further entry criteria were (1) ASA (81–100 mg/daily) for at least 7 days prior to the initial cardiac catheterization and (2) platelet count of 100–350×10^9/L and hemoglobin ≥10 g/dl. Exclusion criteria were: (1) recent bleeding diathesis; (2) hematologic or malignant disorder; (3) oral anticoagulation with coumarin derivatives; (4) glycoprotein IIb/IIIa inhibitor or fibrinolytics administered during either the PCI or the preceding 14 days; and (5) antiplatelet therapy with thienopyridines, cilostazol or dypridimole within the preceding 28 days.

A loading dose of 300 mg clopidogrel was administered on the 1st day, approximately 24 h before PCI. A daily maintenance dose (75 mg) was administered the morning before the procedure and continued thereafter. ASA was administered at a daily dose of 81–100 mg. Blood samples were collected at enrolment, and at 4 (3–5) h, 24 (22–26) h and 48 (46–50) h after the loading dose (**Table 1**). The 24-h sampling was performed before the daily 75 mg clopidogrel intake, whereas the 48-h sampling was afterward. All 30 enrolled patients were to be evaluated until 48 h after the loading dose; 9 patients did not undergo PCI because of unexpectedly mild stenosis and re-evaluation of the PCI indication on the following day and therefore, those patients discontinued clopidogrel intake. PCI was carried out in the remaining 21 patients and their blood samples were analyzed on days 14 (12–16) and 28 (26–30).

Analysis of Platelet Aggregation

Blood samples were collected using a 21G needle, with tourniquet, into a glass tube containing a final solution of 0.313% sodium citrate. Platelet-rich plasma (PRP) was prepared by centrifugation at 150 g at 25°C for 15 min and platelet-poor plasma was prepared by centrifugation at 1,740 g at 25°C for 10 min. The PRPs were stimulated by 5 and 20 μmol/L ADP (adenosine diphosphate; Chronolog), and 2 μg/ml collagen (Horm, Germany) at 37°C and the aggregations were analyzed, under stirring, using a 12-channel light transmission aggregometer (MCM HEMA TRACER 313; MC Medical, Japan), whereby the degree of light transmission of the PRP was defined as 0% of the aggregation rate and the cognitive platelet-poor plasma as 100%.[12,13] The degree of light transmission was monitored for 10 min after agonist stimulation and platelet aggregation was evaluated. All the procedures were completed within 2 h of blood sampling. The maximal aggregation rate (MAR) and the inhibition of platelet aggregation (IPA), which was calculated as the percent inhibition of baseline aggregation according to the following equation, were evaluated:

$$IPA(\%) = \{(MAR_{baseline} - MAR_{time\ after\ treatment})/MAR_{baseline}\} \times 100.$$

Table 1 Study Protocol

		Platelet function analysis
Day 0	*Clopidogrel 300 mg*	*1) Baseline*
		2) 4 h after loading
Day 1	*Clopidogrel 75 mg*	*3) 24 h after loading*
		4) 48 h after loading
	↓	*5) 14 days after loading*
Day 28		*6) 28 days after loading*

Analysis of Vasodilator-Stimulated Phosphoprotein (VASP) Phosphorylation

The VASP is an abundant substrate of cAMP-dependent protein kinase in platelets. Its phosphorylation levels were measured using the Platelet VASP-FCM kit (Biocytex Inc, Marseille, France) in which the VASP-phosphorylation levels are quantified by flow cytometry after stimulation of whole blood with prostaglandin E_1 (PGE_1; mean fluorescence intensity (MFI)) and also PGE_1 plus ADP (MFI PGE_1 + ADP). The $P2Y_{12}$ reactivity index (PRI), calculated as percent inhibition of baseline aggregation was evaluated according to the following equation:

$$PRI = \{(MFI_{PGE1} - MFI_{PGE1+ADP})/MFI_{PGE1}\} \times 100.$$

Table 2 Baseline Characteristics

	Total (n=30)	*PCI (n=21)*	*Non-PCI (n=9)*	*P (PCI vs non-PCI)*
Age (years)	70±7	71±9	68±3	0.11
Males	22 (73%)	17 (81%)	5 (56%)	0.16
Platelets (×10^4/μl)	21.1±5.5	20.4±5.2	22.2±5.8	0.43
Risk factors				
Current smoker	10 (33%)	11 (52%)	0 (0%)	0.0013
Hyperlipidemia[1]	22 (73%)	18 (86%)	5 (56%)	0.083
Diabetes[2]	7 (23%)	6 (29%)	1 (11%)	0.28
Hypertension[3]	18 (60%)	15 (71%)	4 (44%)	0.16
Prior myocardial infarction	1 (3%)	1 (5%)	0 (0%)	0.39
Prior PCI	4 (13%)	3 (14%)	1 (11%)	0.81
Prior cerebrovascular event	1 (3%)	1 (5%)	0 (0%)	0.39
Peripheral vascular disease	2 (7%)	2 (10%)	0 (0%)	0.22
Treatment				
β-blocker	4 (13%)	3 (14%)	1 (11%)	0.81
Nitrates	7 (23%)	5 (24%)	2 (22%)	0.92
ACEI/ARB	13 (43%)	8 (38%)	5 (56%)	0.38
Statin	26 (87%)	19 (90%)	7 (78%)	0.35
Ca-channel blocker	15 (50%)	12 (57%)	3 (30%)	0.23
Proton pump inhibitor	7 (23%)	5 (24%)	2 (22%)	0.92

Defined as [1]under medical treatment or total cholesterol level >220 mg/dl or low-density cholesterol level >140 mg/dl, [2]HbA1c >6.5%, [3]systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg.
PCI, percutaneous coronary intervention; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II-receptor blocker.

Table 3 Maximal Aggregation Rates (%)

	Baseline	*4 h*	*24 h*	*48 h*	*14 days*	*28 days*
ADP (5 μmol/L) stimulation						
Total (n=30)	*64.5±6.7 (30)*	*53.8±10.1 (29)*	*52.9±8.9 (30)*	*49.0±10.2 (28)*		
PCI-treated (n=21)	*65.5±5.9 (21)*	*54.7±10.3 (20)*	*53.6±8.6 (21)*	*49.2±9.8 (20)*	*46.8±12.5 (20)*	*48.8±11.0 (21)*
PCI-untreated (n=9)	*62.0±8.2 (9)*	*52.0±10.0 (9)*	*52.0±10.0 (9)*	*48.6±11.8 (8)*		
ADP (20 μmol/L) stimulation						
Total (n=30)	*72.0±6.2*	*63.1±10.6*	*62.7±10.0*	*59.9±11.1 (28)*		
PCI-treated (n=21)	*72.8±6.5*	*63.8±10.7*	*62.7±9.6*	*60.1±11.4 (20)*	*55.8±10.2 (20)*	*57.0±10.3 (21)*
PCI-untreated (n=9)	*70.0±5.4*	*61.8±8.5*	*62.7±8.5*	*58.4±10.9 (8)*		
Collagen (2 μg/ml) stimulation						
Total (n=30)	*49.3±16.1*	*36.8±16.9*	*36.7±14.2*	*36.1±15.9 (28)*		
PCI-treated (n=21)	*50.3±15.8*	*40.1±16.8*	*38.3±13.2*	*37.7±14.2 (20)*	*33.1±12.2 (20)*	*34.8±11.9 (21)*
PCI-untreated (n=9)	*47.0±17.5*	*29.4±15.3*	*32.8±16.4*	*32.4±17.5 (8)*		

Abbreviation see in Table 2.
The number of examined subjects is shown in parentheses.

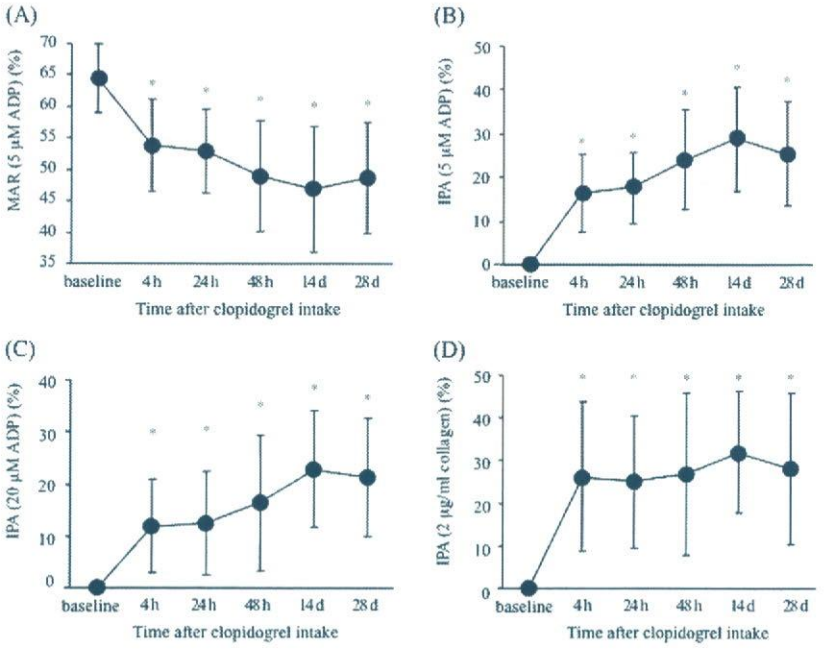

Fig 1. Time-dependent change in platelet aggregation after clopidogrel intake. (**A**) Maximal aggregation rates (MARs) induced with 5 μmol/L ADP. (**B**) Inhibition of platelet aggregations (IPAs) with 5 μmol/L ADP stimulation. (**C**) IPAs with 20 μmol/L ADP stimulation; (**D**) IPAs with 2 μg/ml collagen stimulation. By 1-sample t-test compared with the data at baseline, *P<0.0001.

Definition of Clopidogrel Responsiveness

Classification of clopidogrel effectiveness was based on the definition from a previous report:[14] IPA<10% (clopidogrel non-responders); 10%≤IPA<30% (hypo-responders); IPA≥30% (responders).

Statistic Analysis

Continuous variables are expressed as mean±SD. Categorical variables are expressed as frequencies and percentages. Comparisons between categorical variables were performed using 2-tailed Fisher's exact test or the Pearson's chi-square test. Student's t-test was used to compare continuous variables. Changes in parameters were analyzed using 1-sample t-test. A P-value <0.05 was defined as statistical significance. Statistical analyses were performed using StatView 5.0 software (SAS Institute, Cary, NC, USA).

Results

Characteristics of the Study Population

The baseline characteristics of the 30 enrolled patients are shown in **Table 2**. Mean age was 70±7 years and 22 patients (73%) were male. Only 1 patient (3%) had a history of prior myocardial infarction and 4 (13%) had undergone a prior PCI. Among the 30 patients, 9 did not undergo PCI because of unexpectedly mild stenosis on coronary angiography, which was not apparent on the initial non-invasive assessment. In the others (n=21), PCI with Cypher-stent® implantation was successfully performed. Blood examination was performed until 48 h after intake of 300 mg clopidogrel for all 30 patients, and additionally, on days 14 and 28 post-procedure for the 21 patients undergoing PCI. Because some patients did not cooperate, and other administrative reasons, a few data points were not available. The number of patients evaluated for platelet function was as follows: at 4 h (n=29), 24 h (n=30), and 48 h (n=28) after clopidogrel intake (n=30), and at 14 days (n=20) and 28 days (n=21) among patients undergoing PCI (n=21) (**Table 3**). In all 30 enrolled patients, we did not observe any haematologic disorders or liver dysfunction during the study period.

Baseline characteristics were not significantly different between the PCI (n=21) and non-PCI (n=9) groups, apart from smoking habit (**Table 2**). As shown in **Table 3**, there was no significant difference between the 2 groups in the

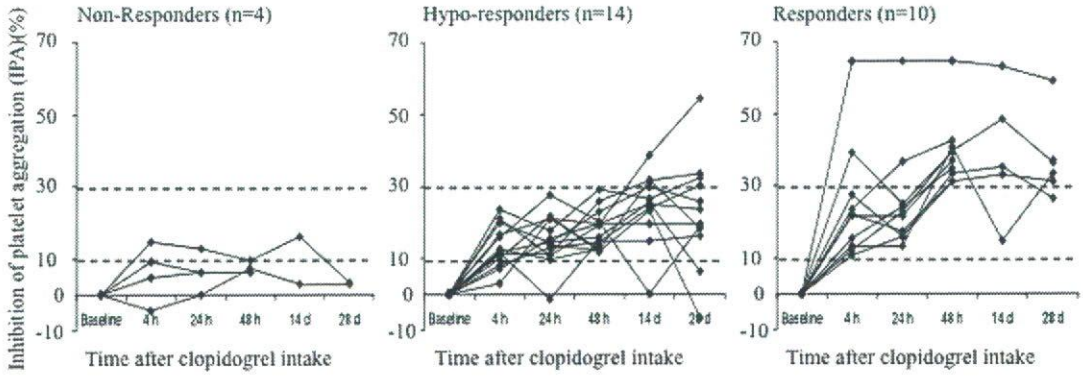

Fig 2. Time-dependent change in the inhibition of platelet aggregation (IPA) after clopidogrel intake for each subject categorized as a responder, hypo-responder or non-responder based on the IPAs with 5 μmol/L ADP stimulation at 48 h after clopidogrel intake, as described in the Methods.

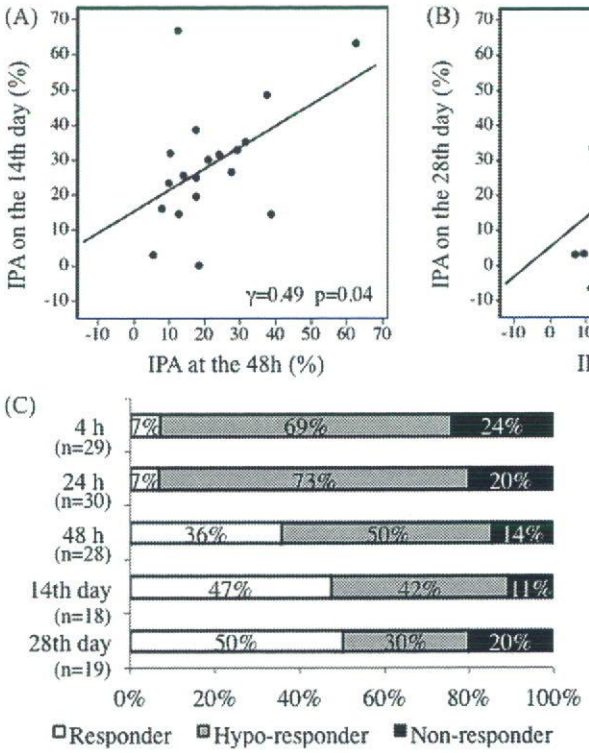

Fig 3. Correlation of the inhibition of platelet aggregation (IPA) with 5 μmol/L ADP at 48 h with values from the 14th day (**A**, n=18) or the 28th day (**B**, n=19) and (**C**) time-dependent change in the ratios of responders, hypo-responders and non-responders at 4 h, 24 h, 48 h, on the 14th day, and on the 28th day after clopidogrel intake using 5 μmol/L ADP as a stimulus are shown according to the definition: IPA <10% as non-responder, 10% ≤IPA <30% as hypo-responder and IPA ≥30%.

ADP-induced MAR at baseline or at 4, 24 or 48 h. Therefore, both groups were analyzed together.

Platelet Aggregation

The MARs induced by 5 μmol/L ADP time-dependently decreased after clopidogrel intake (**Fig 1A**), and the IPA values, which represent the degree of inhibition of platelet aggregability, increased reciprocally (**Fig 1B**). After 300-mg clopidogrel loading, rapid inhibition occurred at 4 h (IPA = 16.4±12.8%, P<0.0001 vs baseline), which continued until 24 h (IPA =17.6±12.1%, P<0.0001 vs baseline). Following 75-mg clopidogrel intake, platelet aggregability was inhibited more intensely after 48 h (IPA =24.0±13.9%, P<0.0001 vs 4 h and P<0.001 vs 24 h). It was noted that IPA did not attain a steady state within 24 h after the initial 300-mg clopidogrel intake. The same trend was observed with 20 μmol/L ADP (**Fig 1C**), for which the IPAs after clopidogrel intake were 11.9±13.6% at 4 h, 12.4±13.9% at 24 h, 16.3±16.3% at 48 h, 22.9±14.5% at 14 days, and 21.3±14.9% at 28 days. These data obtained with 5 or 20 μmol/L ADP stimulation suggest that clopidogrel efficiently exhibited antiplatelet effects and that a 300-mg loading dose might not be immediately sufficient to obtain the maximal antiplatelet effect.

Furthermore, clopidogrel intake also inhibited collagen-stimulated platelet aggregation (**Fig 1D**): IPAs after clopidogrel intake were 26.2±22.4% (4 h), 25.0±19.9% (24 h), 26.8±22.8% (48 h), 31.7±19.0% (14 days), and 29.5±24.9% (28 days).

Rates of Clopidogrel Responders and Non-Responders

We analyzed the inter-individual variation in 5 μmol/L ADP-induced platelet aggregability. Individual plots of the IPAs are shown in **Fig 2**. The effectiveness of clopidogrel exhibited a wide inter-individual variation and was quite constant in individual patients throughout the study period. The effects of clopidogrel were examined on the 14th and

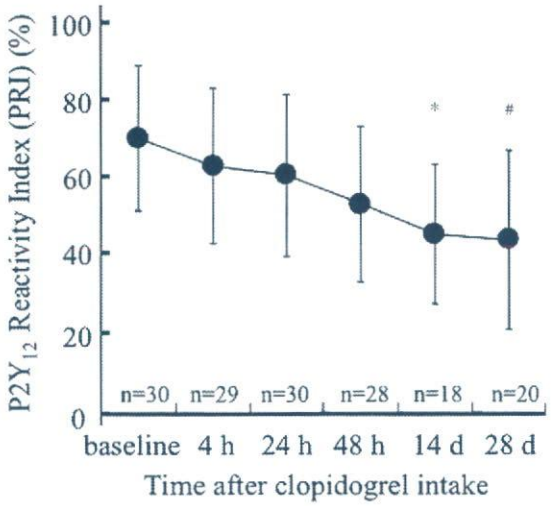

Fig 4. Time-dependent inhibition of P2Y12 reactivity index (PRI), calculated with data based on vasodilator-stimulated phosphoprotein phosphorylation as described in the Methods, at baseline, and at 4 h, 24 h, 48 h, 14th day, and 28th day after clopidogrel intake. By 1-sample t-test compared with data at baseline, *P<0.0001, #P<0.001.

28th days in 21 patients undergoing PCI. Among these patients, the IPAs at 48 h with 5 μmol/L ADP correlated well with those on the 14th day (P=0.04, r=0.49, n=18; **Fig 3A**) and the 28th day (P=0.0007, r=0.71, n=19; **Fig 3B**).

The proportion of responders, hypo-responders, and non-responders at 4 h with 5 μmol/L ADP was 7%, 69%, and 24%, respectively, and 36%, 50%, and 14%, respectively, at 48 h, indicating that the antiplatelet effects of clopidogrel at 48 h were stronger than those at 4 h, although we observed a rapid effect of clopidogrel at 4 h with the 300-mg loading dose. After 48 h, the antiplatelet effects of clopidogrel appeared to reach a plateau (**Figs 1,2**). The rates of non-responders at 48 h, on the 14th day, and on the 28th day were 14%, 11%, and 20%, respectively, while the rates of responders were 36%, 47%, and 50% (**Fig 3C**).

Clopidogrel Responses Evaluated by VASP Phosphorylation

VASP is an abundant substrate of cAMP-dependent protein kinase in platelets. Binding of ADP to P2Y12 leads to G*i*-coupled inhibition of adenylate cyclase, causing reduction of cAMP and the VASP-phosphorylation level in platelets. When P2Y12 receptors are successfully blocked by clopidogrel, the addition of ADP will not reduce the PGE1-indiuced VASP phosphorylation levels. Using these principles, VASP phosphorylation levels were evaluated by flow cytometry in the present study and the PRI was used to evaluate clopidogrel's efficacy: the lower the PRI, the stronger the clopidogrel antiplatelet effect through inhibition of the P2Y12 receptor.

As shown in **Fig 4**, the PRIs gradually decreased after clopidogrel intake in a time-dependent manner: 70.2±19.0% at baseline, 62.9±20.4% at 4 h, 60.4±21.2% at 24 h, 52.9±20.0% at 48 h, 44.9±18.2% on day 14, and 43.8±23.9% on day 28. The PRIs and the IPAs at 48 h after clopidogrel intake were negatively correlated with each other (γ=0.67).

Discussion

In this study, we evaluated the antiplatelet effect of clopidogrel under low-dose ASA therapy in Japanese patients scheduled for PCI, and found that there was a wide inter-individual variation and that the effects in Japanese may not be as strong as for Caucasians at the same dose.

We noted that the effectiveness of clopidogrel was reasonably constant in each patient throughout the study period (**Fig 3**), indicating that responsiveness is individual-specific. In a Western population, the rates of patients with so called 'clopidogrel resistance' ranged between 5% and 44%, although the definitions of clopidogrel resistance varied[9] As shown in **Fig 3**, we also detected 4 (14%) non-responders at 48 h and in 1 patient (3%), clopidogrel suppressed ADP-induced platelet aggregability strongly at 4 h and throughout the study period. These data suggest that there is also a wide variety of responses to clopidogrel in the Japanese.

We used the definition of clopidogrel response proposed by Angiollilo et al because their study design was similar to ours, except that their patients took a higher dose of 250 mg ASA (vs 81–100 mg in our study) and platelet aggregation was evaluated with the optical aggregometer with 6 μmol/L ADP stimulation (vs 5 μmol/L ADP in our study)[14] Therefore, the MAR at baseline in our study (64.5±4.5%) was equivalent to theirs (approximately 60–62%)[14] Importantly, the ratio of responders at 4 h after a 300-mg loading dose was much lower in our study than in their study (7% vs 48%, respectively) and was also the case at 48 h, because the ratios of responders were 36% vs 80%, respectively. Another study conducted in Sweden demonstrated that the mean IPA with 20 μmol/L ADP was approximately 30% at 4 h after a 300-mg loading dose under 325 mg ASA therapy[15] whereas the IPA with 20 μmol/L ADP in our study was 12%. Thus, the degree of platelet inhibition in the Japanese obtained with a similar regimen of clopidogrel, in which a 300-mg loading dose and 75-mg maintenance dose were administered under ASA therapy, might be lower than that in Western populations.

PRI values based on the VASP phosphorylation levels are becoming widely used for the evaluation of the antiplatelet effects of clopidogrel[16,17] We also found them useful because clopidogrel significantly inhibited the PRIs. Using the same loading/maintenance clopidogrel regimen, Grossmann et al report that 10 (17.5%) of 57 patients were inadequate responders (PRI >50%) at 5 days[17] In the present study, the percentages of inadequate responders (RPI >50%) were 16/28 (57%) at 48 h, 10/20 (50%) at 14 days, and 7/21 (33%) at 28 days. Based on these results, we again consider that, at the present dosage, the antiplatelet effect of clopidogrel in the Japanese was not as strong as for Westerners.

Thus, on average, the antiplatelet effects of clopidogrel in Japanese patients are not as strong as those observed in Western people receiving a similar regimen of a 300-mg loading dose followed by a daily 75-mg maintenance dose under ASA therapy. To answer the question whether 75 mg/day clopidogrel is too strong for Japanese, we would answer that, based on the data presented here, it is not the case. Rather, the relatively weaker antiplatelet effect of clopidogrel in Japanese compared with in Western people might cause a higher incidence of stent thrombosis. However, currently we have no data on the degree of antiplatelet effect by clopidogrel that is necessary for the prevention of stent thrombosis in Japanese patients. Furthermore, because little data are available concerning the effect of ticlopidine in Japanese that would be sufficient to prevent stent thrombosis, we cannot conclude that the antiplatelet effect of clopidogrel at the current dosage is insufficient to prevent stent thrombosis. Further study is essential to link the effec-